Exploring the drivers of European native oyster (*Ostrea edulis*) phenology in the German Bight

Untersuchung der Einflussfaktoren der Phänologie der Europäischen Auster (Ostrea edulis**) in der Deutschen Bucht**



The School of Mathematics and Science of the University of Oldenburg,
has accepted as partial fulfilment of the requirements
for the degree and title of
Doctor of Philosophy
- Ph.D. -
the above-mentioned thesis by

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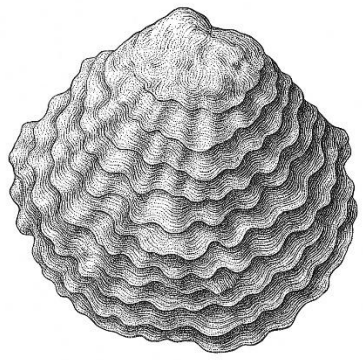
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Date of thesis defence: 08.12.2025

To my parents and my sisters



Aknowledgment

A PhD may look like a solitary marathon, but in truth it is more of a collective expedition. This thesis would not have been possible without the support, advice, and encouragement of many people along the way, and so now I want to thank them.

First, I would like to thank my supervisor, **Bettina**, for welcoming me to AWI and into your team. Thank you for your advice and for allowing me to carry out this project with both independence and guidance, which enabled me to complete it successfully, producing solid results and publications. I will also keep good memories of sharing moments outside the lab, such as make you discover Arcachon and Bordeaux.

I am also grateful to my co-supervisors, **Damien** and **Laura**, for supporting me since my first Master's internship and for introducing me to chronobiology, a field I truly enjoy. Your feedback (and patience) on writing, your scientific input, the long nights at the marine station for 24-hour samplings, and your help during Helgoland fieldwork, even under the snow and wind of the North Sea, were essential to the success of this project and are among the best memories of my PhD.

My other co-supervisor, **Bernadette**, thank you for your warm welcome into the Helgoland team, and for extending my contract just long enough to finish this project without losing all my hair. I also thank you for including me in the NORA network, which gave me the opportunity to present our results at the Cartagena conference and meet passionate people working on flat oyster.

To my thesis committee: **Kristin**, thank you for your thoughtful scientific questions, which pushed me to reflect more deeply on chronobiology and molecular biology, and for taking the time to evaluate this dissertation. **Stéphane**, your enthusiasm, curiosity, and deep knowledge of these fascinating species made every meeting not only productive but also inspiring. Our discussions on oyster spawning led to a very exciting paper, and I hope that our discoveries on flat oysters will continue beyond this thesis.

To Bettina's group: **Lukas**, thank you for showing me around Oldenburg when I first arrived in Germany and for hosting me during the Oldenburg conference, also thank for your help with the light sensor, and for your inspiring motivation and kindness. I wish you all the best for the completion of your thesis. **Svenja** and **Dominik**, thank you for always being ready to help with both curiosity and good mood. I hope you will find future projects that truly inspire you, because you deserve it. And of course, I would be delighted to welcome you in Brittany if my adventure continues in Brest. **Kerstin**, thank you for your assistance in the lab, for always helping me whenever I had problems with machines, and for making the lab a nicer place with your smile and your playlists, which also improved my German music culture.

To the Helgoland team: **Bérenger**, thank you for our discussions and your passion about oysters, I wish you great adventures at Thünen, and maybe see you again in Normandy or Brittany. **Julius**, thank you for your always kind feedback after my presentations and it was a pleasure to meet you at ITRS and to represent Helgoland at this international congress. **Nico**, **Martin**, **Nick**, **Henning**, **Corina**, and **Lennard**, your good mood and always interesting conversations made the deployment of the experiment a success in a positive atmosphere. **Celina**, my partner in crime for the cleaning of 1,000 oysters and the creation of a homemade frame system (which impressively lasted 14 months!), we truly

made a very efficient duo. To the diving team, **Laura**, **Miri**, **Markus**, **Phillip**, and all the divers who participated during the monthly samplings, your professionalism and flexibility made these monthly samplings possible, even if their success sometimes felt uncertain under such challenging weather conditions. Your good mood on the boat made all the difference. **Anna**, thank you for every warm welcome, every smile, and your enthusiastic interest in the project. **Uwe**, thank you for always being available when I needed help. **Kathrin**, **Regina**, and **Lenka**, your support during my stays on the island made everything much easier.
To the AWI people, thank you to everyone I met during lunch breaks or during Science Weeks, it was always inspiring to hear about your research topics. In particular, **Morten** and **Tanja**, thank you for your warm welcome into the section. **Sören**, thanks for our discussions about chronobiology and for sharing your office with me during my time at AWI (before that the quarantine area separated us).
To the Arcachon marine station: **Alexia**, thank you for welcoming me into the team. **Audrey**, without your LED expertise, I would still be stuck in the dark, both literally and scientifically. Thanks a lot for your support as an office neighbor, and for sharing the joys (and frustrations) of SNCF train delays and cancellations. I wish you all the best in your new life in Nantes, you deserve it. **Yannick**, your patience with technical questions, your calm presence during deployments in Helgoland, and your playlists for blind tests after long field days were priceless. **Emilie**, I appreciated our office discussions, your stories from missions, and our healthy lunches with "l'équipe du gras". Thanks also to the two **Marine** for sharing your office down the hall for some time, it was great to support each other in the different stages of our PhDs, I wish you the best for your next steps. **Laure**, thank you for guiding me through the jungle of phylogenetic trees. Speaking of phylogeny, I would also like to thank **Christophe Klopp** (INRAE) for his valuable help with the bioinformatics and data mining work. **Flore** and **Cerise**, thank you for your important laboratory contributions, especially for the labial palps work that allowed me to go to Helgoland in time for the start of the field experiment. **Christelle**, our fun breaks kept me sane between qPCR runs. And to the broader Arcachon group: **Fabien**, **Patrice**, **Deborah**, **Yann**, **Leslie**, and everyone else from the marine station, thank you for lively lunch debates and cheerful Christmas meals.

On a personal note, these three years between France and Germany would not have been possible without the support of my friends and family.

In the south of France: **Valou** and **Ziol** thank you for our many dinners, soundsystem and vineyards visits and for our weekly climbing-and-sauna ritual with **Valou**, I look forward to more adventures together. **Blandine** and **Micka**, thank you for the ski trip, rugby games at Chaban-Delmas, and especially thank you for including me in the construction work on your new house, wheelbarrow rounds were a kind of physical therapy, giving me a break and a way to think about something other than thesis writing. **Alice** and **Greg**, the "Montpelliérains", your support and enthusiasm (including your excitement about traveling to my defense from the very beginning) meant the world, thank you also for our great ski and Bordeaux trips, I hope many more will come. **Thomas** and **Paula**, the Master2 "triforce": we started our PhDs together and reached the finish line side by side. Meeting again in Brest for the congress after these three years was a highlight, and I hope our adventures and reunions will continue.

In the north of France, in Brittany, my refuge: **Manue** and **Sten**, your kind words and support have meant a lot, so much has happened over these years, from supporting me as two to now as a family of three. I can't wait to return to Brittany and spend more time together. **Ingrid** and **Théo**, our New Year parties, festival and beach picnics (always with red Kasteel) gave me energy and joy. My "brother from another mother" **Théo**, thanks for the Twitch catch-ups, our slow texting pace but our reunions every time I returned to Brittany. **Flo**, from high school through our biology degree and up to now, you've become a bat-man, I an oyster-man, thank you for always being there along the way.

In the North, but this time in Germany, in Bremerhaven: my thanks to **Pedro** for your welcome in our shared flat "Der Walde", for hockey games, and for debates on Spanish and French politics. Thanks also to **Simone** and **Eli**, my French flatmates, for our never-ending conversations about cinema, manga, politics, and for supporting each other through different stages of our PhDs at AWI, that mental support was important. And to the many others who shared beers in local bars or Glühwein at the Christmas market, thank you for making this cold northern city feel warm.

Finally, to **my parents**: thank you for your loving and unwavering support, even when my academic path was uncertain. Thank you for being my everyday inspiration, **dad**: listening to your Antarctic expedition stories surely planted the seed that gave me a taste for discovery and adventures, which is why reaching the end of this journey to become a marine biologist by being in a polar institute makes all the more special (even if Helgoland was very far from the polar conditions described by my AWI colleagues). **Mom**, you are my strength every day. To **my sisters**: though distance sometimes makes us slow texters, every reunion in Vannes, Lille, Bordeaux, Madrid, or Barcelona has been precious. I love you, and I will always be there for you, as you have been for me during this adventure. To **my large family**, uncles, aunts, cousins, thank you for the laughter, holidays, and Christmas celebrations together that always recharges me. To my "**belle-famille**," thank you for the Christmases, summers, birthday celebrations, and Moni's volleyball matches in Bordeaux. In three years, so much has happened, including the arrival of two little nieces/nephews who happily kept us busy, the perfect way to burn off energy and temporarily escape from thesis work.

And last but not least, thanks to **Mélanie**. For eight years you have been my constant, my listener (even through my monologues about oysters) and my partner in adventures. Thank you for sharing your passions with me and for always joining me in activities that weren't always your own, knowing how much they meant to me whenever I was back in France, for instance, traveling to Toulouse just to watch a rugby match (and to enjoy real cassoulet and local specialties, which, in this case, is a common passion). I am very a lucky guy to have you on my side. Also thank you for visiting me in Bremerhaven, Hamburg, and Helgoland, and discovering this part of my life. Finally, thank you for your patience, your love, and your support to follow me through the tides of this thesis. I look forward to the next adventures together, and wherever the next chapter takes us, Brest, Nantes, or Vannes, I know that I will already feel at home, because you will be there.

Table of Contents

Summary 3
Zusammenfassung 5
List of Abbreviation 8
1. Introduction 9
1.1 The European flat oyster, *Ostrea edulis* 9
1.1.1 Biology, historical distribution and taxonomy 9
1.1.2 Anatomy and reproductive cycle 11
1.1.3 History of the flat oyster and aquaculture 15
1.1.4 Focus on the German Bight and Helgoland 17
1.2 Re-introduction of the species 20
1.2.1 Recent re-introduction efforts 20
1.2.2 Oyster reef re-introduction benefits 21
1.3 Biological rhythms 22
1.3.1 Definition 22
1.3.2 Short history 23
1.3.3 Rhythm characteristics 24
1.3.4 The circadian rhythm 26
1.3.5 The circannual rhythm 27
1.3.6 The molecular clock 28
1.4 Biological rhythms in marine organisms 31
1.4.1 The coastline ecosystem: A highly variable habitat 31
1.4.2 Chronobiology in marine organisms 34
1.4.3 Impacts of global environmental change on marine rhythmicity 36
1.5 Research objectives of the thesis 37
1.6 Scopes and structure of the dissertation 38
1.6.1 Article I (Task #2) - Characterization of the daily and circadian valve behavior 38

1.6.2 Article II (Task #1) – Identification and expression of circadian clock genes 39
1.6.3 Article III (Task #3 and Task #4) - Phenology of *Ostrea edulis* and its environmental drivers....40
1.6.4 Article IV (Task #3) – *Ostrea edulis* valve behavior during reproduction events 41
2. List of Manuscripts....43
3. Article I - Characterization of the daily and circadian valve behavior....45
4. Article II - Identification and expression of circadian clock genes....67
5. Article III - Phenology of *Ostrea edulis* and its environmental drivers....99
6. Article IV - *Ostrea edulis* valve behavior during spawning events....143
7. Discussion and perspectives....173
7.1 Advances in the understanding of chronobiology of *Ostrea edulis*....175
7.1.1 Behavioral rhythms and endogenous control....175
7.1.2 Molecular clockwork identification and temporal gene expression....177
7.2 Advances in the understanding of *Ostrea edulis* behavior and response to environmental parameters....178
7.3 Contribution to the understanding of *Ostrea edulis* reproduction....180
7.4 Integrative perspectives: linking behavior, molecular clocks, and reproduction...181
7.5 Conclusion and perspectives for restoration and reintroduction projects....183
7.5.1 Implications for restoration and reintroduction....184
7.5.2 Open questions and future directions....185
8. References cited in Introduction and Discussion....186
9. Appendix....207
9.1 Supplementary material for Article I....207
9.2 Supplementary material for Article II....215
9.3 Supplementary material for Article III....222
9.4 Supplementary material for Article IV....229
Erklärung der Autorin....237

Summary

The European flat oyster (*Ostrea edulis*) is a keystone species of coastal ecosystems and an important ecosystem engineer. Once widespread in Europe from the Mediterranean to the North Sea, it has been a major food source since the Middle Ages. Overfishing and habitat destruction led to its near extinction in large parts of Europe. Today, *O. edulis* is a focus of oyster reef habitat restoration programs. The recovery of this species and its associated biocenosis will require decades-long efforts. For the long-term success, a thorough understanding of its resilience to environmental variability and climate change is crucial.

Biological rhythms, driven by endogenous clocks, are central to the regulation of life-cycle traits such as growth, reproduction, and behavior. These rhythms are synchronized with geophysical cycles linked to the sun, earth, and moon. While chronobiology has been extensively studied in terrestrial model organisms, little is known about endogenous clocks in marine invertebrates, and almost nothing is known for *O. edulis*. Endogenous clocks may be key to anticipating and adapting to predictable cycles, but also a vulnerability under rapid anthropogenic change.

Despite its ecological importance and relevance for conservation, fundamental knowledge on the biology and chronobiology of *O. edulis* is lacking. First, no experimental evidence existed regarding whether this species possesses a functional endogenous circadian clock, nor how this potential clock regulates daily behavior. Second, the molecular basis of timekeeping in *O. edulis* had not been identified, leaving an important gap in understanding the genetic and evolutionary underpinnings of its rhythmicity. Third, while restoration programs progress rapidly, there was no integrative knowledge of oyster rhythms in natural habitats, particularly regarding how valve behavior, growth, and molecular oscillations are synchronized with environmental cycles. Finally, the temporal organization of reproduction in *O. edulis*, including spawning, incubation, swarming, and sex change, remained poorly documented, particularly under natural conditions.

This dissertation addresses these gaps by combining laboratory and field approaches to:
(i) determine whether *O. edulis* possesses a functional circadian clock;
(ii) identify and characterize its molecular clock components;
(iii) investigate behavioral, growth, and molecular rhythms under natural field conditions; and (iv) describe the reproductive timing and sex-specific behaviors of this species.

In **chapter 3 (Article I)**, I provide the first chronobiological characterization of *O. edulis* behavior under laboratory conditions. Using non-invasive valvometry, we demonstrate that the flat oyster possesses a functional circadian clock generating rhythmic valve activity under free-running conditions. These rhythms are plastic, entrainable by light cues, and disrupted by

artificial light at night, highlighting both the adaptive potential and the vulnerability of the oyster's timing system to anthropogenic pressures.

In **chapter 4 (Article II)**, I characterize the molecular clockwork of *O. edulis*. Phylogenetic and domain analyses revealed orthologs of all major circadian clock genes as well as genes involved in photoreception and melatonin synthesis. Tissue-specific expression analyses demonstrated rhythmic transcription under light:dark and constant conditions, confirming the presence of a functional and plastic endogenous circadian system. By linking molecular oscillations to behavioral outputs, this chapter establishes the genetic foundation for understanding timekeeping in *O. edulis* and provides essential resources for future chronobiological and restoration research.

In **chapter 5 (Article III)**, I expand to the field, integrating behavioral, growth, and molecular data collected over 540 days on Helgoland. Using high-resolution valvometry, shell growth monitoring, and monthly gene expression profiling, we show that *O. edulis* exhibits multiple rhythms across daily, tidal, lunar, semilunar, and annual scales under field conditions. These rhythms are coordinated primarily by photoperiod, but also modulated by temperature, underwater sound and food availability. This chapter demonstrates the complexity and plasticity of oyster chronobiology *in situ*, while highlighting its sensitivity to environmental perturbations. These findings provide crucial ecological context for conservation efforts.

In **chapter 6 (Article IV)**, I investigate reproductive rhythms and reveal, the valve behavior associated with spawning in both sexes, as well as larval incubation and larval swarming behaviors in females. Our findings show a high degree of synchronization between male and female spawning events, suggesting coordinated reproductive timing. I also demonstrate that these behaviors are influenced by environmental drivers such as photoperiod, lunar and tidal cycles, as well as temperature fluctuations. This work provides novel insights into the temporal organization of reproduction and sex change in O. edulis, underlining the central role of biological timing in its reproductive ecology and highlighting key considerations for restoration and population management.

In conclusion, this dissertation provides the first comprehensive chronobiological framework for *O. edulis*. By linking molecular clocks to behavior, growth, and reproduction across laboratory and field settings, it uncovers a multiple rhythmic system that underpins ecological adaptation but is vulnerable to anthropogenic stressors such as climate change and light pollution. These findings contribute critical knowledge to oyster biology and restoration ecology, offering mechanistic insights into oyster resilience and informing predictive models for conservation and management under global change.

Keywords: *Ostrea edulis*, phenology, restoration, valve behavior, biological rhythms

Zusammenfassung

Die Europäische Auster (*Ostrea edulis*) ist eine Schlüsselart und ein wichtiger Ökosystemingenieur europäischer Küstenökosysteme. Einst vom Mittelmeer bis in die Nordsee weit verbreitet, war sie seit dem Mittelalter eine wichtige Nahrungsquelle. Überfischung und Lebensraumzerstörung führten in weiten Teilen Europas zur fast vollständigen Ausrottung. Heute steht *O. edulis* im Mittelpunkt großer Wiederherstellungsprogramme für Austernriffhabitate. Die Erholung dieser Art und ihrer assoziierten Biozönose erfordert jahrzehntelange Anstrengungen. Für den nachhaltigen Erfolg von Wiederherstellungsmaßnahmen ist ein umfassendes Verständnis ihrer Resilienz gegenüber Umweltveränderungen und dem Klimawandel entscheidend.

Biologische Rhythmen, die von endogenen Uhren gesteuert werden, spielen eine zentrale Rolle bei der Regulierung von Lebenszyklusaspekten wie Wachstum, Fortpflanzung und Verhalten. Diese Rhythmen sind mit geophysikalischen Zyklen synchronisiert, die mit Sonne, Erde und Mond zusammenhängen. Während die Chronobiologie bei terrestrischen Modellorganismen umfassend untersucht wurde, ist über endogene Uhren bei marinen Wirbellosen nur wenig, und über *O. edulis* fast nichts bekannt. Endogene Uhren sind entscheidend für die Anpassung an vorhersehbare Zyklen, sind aber auch eine potenzielle Schwachstelle bei der Anpassung an anthropogen verursachte Veränderungen.

Trotz ihrer ökologischen Bedeutung und ihrer Relevanz im Naturschutz fehlen grundlegende Kenntnisse über die Biologie und Chronobiologie von *O. edulis*. Erstens gab es keine experimentellen Belege dafür, dass diese Art über eine funktionierende endogene circadiane Uhr verfügt, noch dafür, wie diese potenzielle Uhr das tägliche Verhalten reguliert. Zweitens war die molekulare Grundlage der Zeitmessung bei *O. edulis* nicht identifiziert, eine wichtige Lücke im Verständnis der genetischen und evolutionären Grundlagen ihrer Rhythmik. Drittens gab es trotz fortschreitenden Wiederherstellungsprogrammen kein integratives Wissen zu endogenen Rhythmen von Austern in ihrem natürlichen Lebensraum, insbesondere wie Verhalten, Wachstum und molekulare Oszillationen mit Umweltzyklen synchronisiert sind. Detailkenntnisse zum zeitlichen Ablauf der Fortpflanzung bei *O. edulis*, Laichen, Inkubation und Geschlechtsumwandlung, gab es kaum.

Diese Dissertation befasst sich mit diesen Wissenslücken, indem sie Labor- und Feldansätze kombiniert, um:

(i) festzustellen, ob *O. edulis* über eine funktionale circadiane Uhr verfügt;

(ii) die molekularen Uhrkomponenten zu identifizieren und zu charakterisieren;

(iii) Verhaltens-, Wachstums- und molekulare Rhythmen unter natürlichen Feldbedingungen zu untersuchen;
(iv) den Zeitpunkt der Fortpflanzung und geschlechtsspezifische Verhaltensweisen dieser Art zu beschreiben.

In **Kapitel 3 (Artikel I)** liefere ich die erste chronobiologische Charakterisierung des Verhaltens von *O. edulis* unter Laborbedingungen. Mithilfe der nicht-invasiven Valvometrie zeigen wir, dass die heimische Europäische Auster über eine funktionierende circadiane Uhr verfügt, die unter freien Bedingungen eine rhythmische Klappenaktivität erzeugt. Diese Rhythmen sind plastisch, können durch Lichtreize synchronisiert werden und werden durch künstliches Licht in der Nacht gestört, was sowohl das Anpassungspotenzial als auch die Anfälligkeit des Zeitmesssystems der Auster gegenüber anthropogenen Einflüssen unterstreicht.

In **Kapitel 4 (Artikel II)** charakterisiere ich das molekulare Uhrwerk von *O. edulis*. Phylogenetische und Domänenanalysen ergaben Orthologe aller wichtigen circadianen Uhrgene sowie Gene, die an der Photorezeption und Melatoninsynthese beteiligt sind. Gewebespezifische Expressionsanalysen zeigten eine rhythmische Transkription unter Licht-Dunkel- und konstanten Bedingungen, was das Vorhandensein eines funktionellen und plastischen endogenen circadianen Systems bestätigt. Durch die Verknüpfung molekularer Oszillationen mit Verhaltensausprägungen legt dieses Kapitel die genetische Grundlage für das Verständnis der Zeitmessung bei *O. edulis* und liefert wichtige Ressourcen für die zukünftige chronobiologische und restaurative Forschung.

In **Kapitel 5 (Artikel III)** erweitere ich den Blickwinkel auf das gesamte Gebiet und integrieren Verhaltens-, Wachstums- und Molekulardaten, die über einen Zeitraum von 540 Tagen auf Helgoland gesammelt wurden. Mithilfe hochauflösender Valvometrie, Überwachung des Schalenwachstums und monatlicher Genexpressionsprofilierung zeigen wir, dass *O. edulis* mehrere Rhythmen auf täglicher, gezeitenabhängiger, monatlicher, halbmonatlicher und jährlicher Ebene aufweist. Diese Rhythmen werden in erster Linie durch die Photoperiode und die Temperatur koordiniert, aber auch durch Unterwassergeräusche und die Verfügbarkeit von Nahrung moduliert. Dieses Kapitel demonstriert die Komplexität und Plastizität der Chronobiologie von Austern *in situ* und hebt gleichzeitig ihre Empfindlichkeit gegenüber Umweltstörungen hervor. Diese Erkenntnisse liefern einen wichtigen ökologischen Kontext für Naturschutzmaßnahmen.

In **Kapitel 6 (Artikel IV)** untersuche ich Fortpflanzungsrhythmen und zeigen das mit dem Laichen verbundene Verhalten zur Schalenöffnung beider Geschlechter sowie die Larveninkubation- und -entlassung der Weibchen. Unsere Ergebnisse zeigen einen hohen Grad an Synchronisation zwischen den Laichereignissen von Männchen und Weibchen, was auf einen

koordinierten Fortpflanzungszeitpunkt hindeutet. Wir zeigen auch, dass diese Verhaltensweisen durch Umweltfaktoren wie Mond- und Gezeitenzyklen sowie Temperaturschwankungen beeinflusst werden. Diese Arbeit liefert neue Erkenntnisse zur zeitlichen Organisation der Fortpflanzung und Geschlechtsumwandlung bei *O. edulis*, unterstreicht die zentrale Rolle des biologischen Zeitplans in seiner Fortpflanzungsökologie und hebt wichtige Überlegungen für die Wiederherstellung und das Populationsmanagement hervor.
Zusammenfassend lässt sich sagen, dass diese Dissertation den ersten umfassenden chronobiologischen Rahmen für *O. edulis* liefert. Durch die Verknüpfung molekularer Uhren mit Verhalten, Wachstum und Fortpflanzung in Labor- und Feldversuchen deckt sie ein multiples rhythmisches System auf, das die ökologische Anpassung unterstützt, aber anfällig für anthropogene Stressfaktoren wie Klimawandel und Lichtverschmutzung ist. Diese Erkenntnisse liefern wichtige Erkenntnisse für die Renaturierungsökologie, bieten mechanistische Einblicke in die Widerstandsfähigkeit von Austern und liefern Informationen für Vorhersagemodelle für den Schutz und das Management unter globalen Veränderungen.

Schlüsselwörter: Ostrea edulis, Phänologie, Renaturierung, Klappenverhalten, biologische Rhythmen

List of Abbreviation

AIC	Akaike Information Criterion
ALAN	Artificial Light At Night
AWI CSD	Alfred Wegener Institute Centre for Scientific Diving
Blastp	Protein-protein Blast
ccg	Clock controlled genes
cDNA	complementary Deoxyribonucleic Acid
CDS	Coding sequence
Chl *a*	Chlorophyll *a*
CT	Circadian time
D:D or DD	Dark:Dark
EU	Experimental unit
FM	Full moon
FQM	First quarter moon
HFNI	High-frequency non-invasive
LED	Light-emitting diode
L:D or LD	Light:Dark
L:L or LL	Light:Light
LQM	Last quarter moon
LMMs	Linear mixed-effects models
LS	Lomb and Scargle
Lx	Lux
ML	Maximum likelihood
NM	New moon
NORA	Native Oyster Restoration Alliance
NSTC	Neap spring tidal cycle
OSPAR	Oslo-Paris commission
PAR	Photosynthetically Active Radiation
PR	Percent Rhythm
TTFL	Transcription-translation feedback loop
UTC	Coordinated universal time
VOA	Valve Opening Amplitude
VOD	Valve Opening Duration
ZT	*Zeitgeber* time

1. Introduction

1.1 The European flat oyster, *Ostrea edulis*

1.1.1 Biology, historical distribution and taxonomy

The European native oyster or flat oyster, *Ostrea edulis* (Linnaeus, 1758), is a bivalve mollusc of the Ostreidae family (Table 1.1). Bivalves are an ancient lineage that has persisted for more than 400 million years, with the earliest oyster fossils dating back ~200 million years (Wanninger & Wollesen, 2019). Among these, *Ostrea edulis* is a filter-feeding species, primarily consuming phytoplankton suspended in the water column.

Table 1.1. Taxonomic tree of *Ostrea edulis* (Linnaeus, 1758)

Domain:	Eukaryota
Kingdom:	Metazoa
Phylum:	Mollusca
Class:	Bivalvia
Subclass:	Pteriomorphia
Order:	Ostreoida
Family:	Ostreidae
Genus:	Ostrea
Species:	*Ostrea edulis*

Its historical native range spans from the Norwegian Sea in the north, encompassing the coastal waters of Ireland and Britain, the Iberian Peninsula, and extending into the Mediterranean Sea in the south (Beck et al., 2011; Thurstan et al., 2023) (Figure 1.1). It typically inhabits relatively sheltered coastal environments such as bays and estuaries in waters less than 10 meters. However, historical accounts also document the presence of dense natural oyster beds in subtidal coastal waters as well as deeper offshore areas reaching depths of up to 80 meters, particularly in regions such as the North Sea and the eastern English Channel (Olsen, 1883; Pogoda, 2019; Preston et al., 2020; zu Ermgassen et al., 2025).

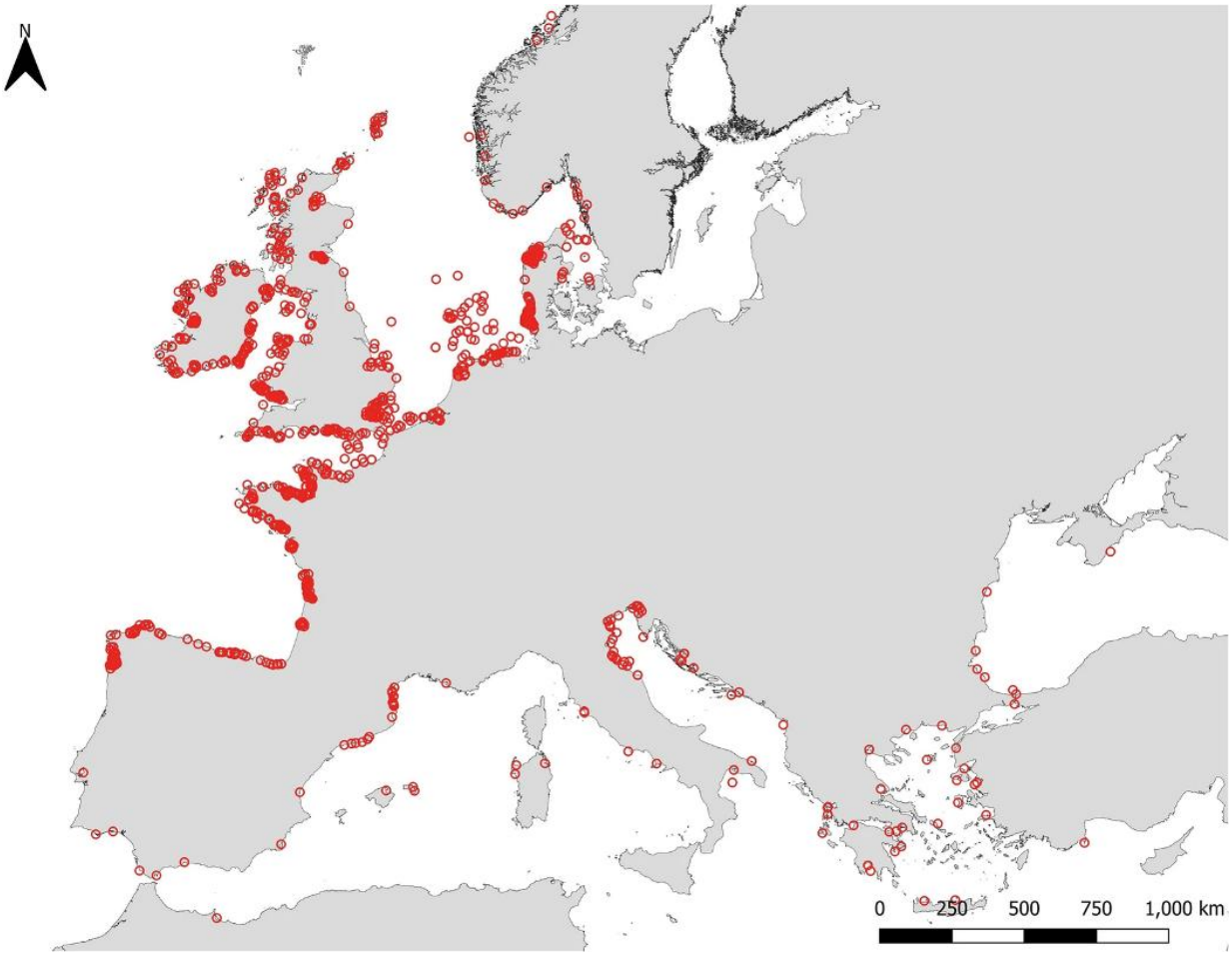


Figure 1.1: Distribution, extent, and form of lost *Ostrea edulis* reef ecosystems in Europe, based on historical sources (from Thurstan et al., 2024).

The life cycle of *O. edulis* begins with a planktonic larval stage. After a brief free-swimming period, the larvae settle on hard substrates, ranging from intertidal zones to subtidal depths, where they permanently attach themselves and develop into benthic juveniles and adults (Bayne, 1969; Lapegue et al., 2006). As a gregarious reef-forming species, juvenile oysters preferentially settle on existing shells or living conspecifics (Bayne, 1969). Over successive generations, these aggregations build up into complex, three-dimensional reef structures composed of layered oyster shells (Figure 1.2). These biogenic reefs provide essential habitat complexity and play a significant ecological role in coastal ecosystems (Haelters & Kerckhof, 2009; zu Ermgassen et al., 2016).



Figure 1.2. Example of oyster clump in the Bay of Quiberon, France. Photo: S.Pouvreau / Ifremer.

1.1.2 Anatomy and reproductive cycle

Ostrea edulis is a sessile, asymmetrical bivalve with two calcified valves joined by a hinge and ligament (Bayne, 2017). The left valve is cemented to a hard substrate, usually during the post-larval stage, via a secretion from the mantle (Bayne, 1969; Cole, 1938). The right valve adapts in shape accordingly to ensure a watertight closure. Shells are mainly composed of calcite, with aragonite concentrated around the adductor muscle and ligament insertions (Kızılkaya et al., 2024). They can reach up to 20 cm and show high morphological variability, reflecting a response to environmental constraints such as crowding within reefs or substrate shape (Campbell, 2012). Their general anatomy shown in Figure 1.3, have been described in details by (Yonge, 1926). It is closely similar that of the Pacific oyster *Crassostrea gigas*, for which more detailed functional studies are available. Thus, in several cases, functional explanations of tissues will refer to insights gained from studies on *C. gigas*.

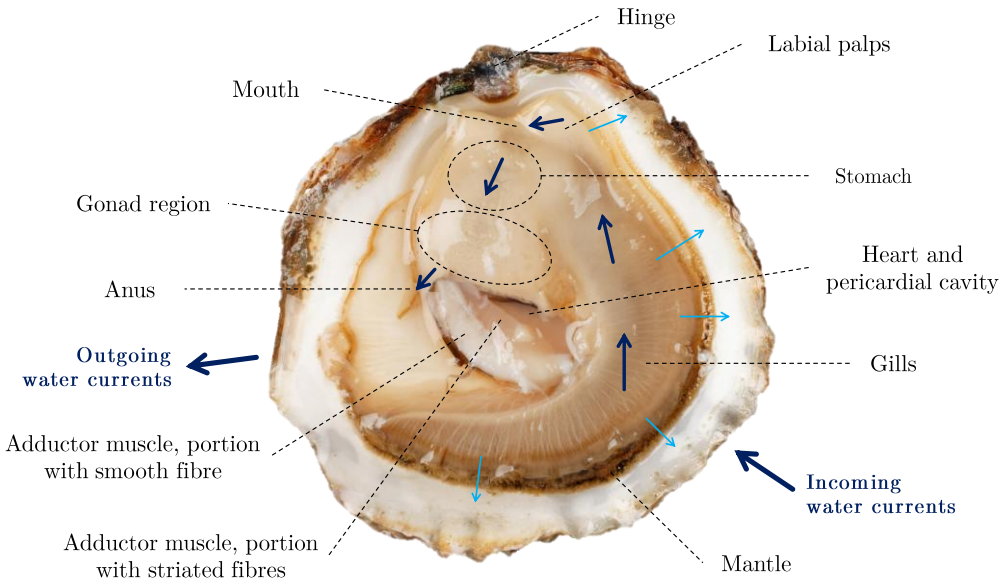


Figure 1.3. Anatomy of *Ostrea edulis*. Large dark blue arrows outside the shell indicate the direction of incoming and outgoing water currents. Inside the shell, dark blue arrows indicate the main water currents, encompassing both inflow and outflow, while thinner light blue arrows represent secondary outgoing currents (adapted from Yonge (1926), Photo: S.Deroire).

Shell development and valve movement

The mantle (Figure 1.3), a soft tissue layer lining the inner shell, plays a central role in shell secretion and maintenance (Jodrey, 1953). It also contributes to sensory perception via nerve endings and produces nacre for internal shell lining. Valve movement is controlled by a central adductor muscle, composed of two distinct regions (Figure 1.3): a smooth muscle for prolonged closure at low energetic cost, and a striated muscle for rapid reflexive responses (Galler et al., 2009; Yonge, 1926).

Respiration and feeding

In *O. edulis*, respiration and food capture occur through the gills (Figure 1.3), which consist of two pairs of pseudolamellibranch lamellae perforated by ostia through which water flows (Yonge, 1926). The gills serve both respiratory and feeding functions: haemolymph within the filaments facilitates gas exchange (Gosling, 2003; Yonge, 1926), while a complex arrangement of cilia enables food capture and transport. Lateral cilia generate water currents; latero-frontal and frontal cilia trap and convey particles toward

the labial palps (Gosling, 2003; Yonge, 1926). The gills extend in a semicircle from the posterior junction of the mantle folds to the labial palps (Figure 1.3), partially enclosing the gill filaments at their anterior ends (Yonge, 1926). Labial palps, arranged in two pairs (four in total), sort the particles before ingestion (Beninger et al., 1995). These are triangular flaps with ridged inner surfaces bearing bands of long cilia, which beat in various directions to facilitate sorting. Excess or unsuitable particles are expelled as pseudofeces. Filtration efficiency is regulated by modulating ciliary activity and ostia size, and varies with environmental factors such as temperature, salinity, and suspended particle load (Gosling, 2003; Yonge, 1926).

The digestive system includes the mouth, esophagus, stomach, digestive gland, intestine, and anus (Figure 1.3). The mouth, enclosed by the fused bases of the labial palps, opens as a horizontal slit and leads into a short esophagus, which passes into the stomach. A crystalline style in the stomach aids in mechanical and enzymatic digestion by releasing enzymes upon food arrival and reforming after digestion (Morton, 1977). Waste is expelled into the exhalant water stream through the anus, which opens on a papilla at the posterior margin of the adductor muscle (Yonge, 1926).

Circulatory and nervous system

The circulatory system is semi-open. A muscular heart (Figure 1.3), consisting of a ventricle and two auricles, pumps haemolymph through the arterial system to the kidneys and gills for oxygenation, before returning to the heart (Yonge, 1926). The nervous system includes visceral ganglia near the adductor muscle, cerebral ganglia at the base of the labial palps, and peripheral nerves innervating tissues like the gills and mantle.

Reproductive cycle

O. edulis is a sequential hermaphrodite, generally maturing first as a male at 2-4 years of age, then alternating between sexes (Cole, 1942; Orton, 1927; Walne, 1974). Sexual development is temperature-dependent, typically requiring water temperatures above 15-17°C for spawning. A key reproductive distinction between Ostrea and Crassostrea genus is larviparity: in O. edulis, fertilization occurs internally and embryos are brooded within the female gills until the larval stage (Figure 1.4), whereas in Crassostrea species

fertilization is external and gametes are released directly into the water column (Bayne, 2017). Gametes develop seasonally in the gonad (Figure 1.3), which forms around the digestive gland during gametogenesis and regresses afterward. During reproduction, males release spermatozoa into the water column in the form of spherical spermatozeugmata (Suquet et al., 2018), which are subsequently taken up by females via filtration. Fertilization occurs within the female's mantle cavity, followed by internal brooding of larvae for 5-12 days until they reach 120-160 µm (Gray et al., 2022; Korringa, 1940). Once developed, larvae are released into the plankton where they feed on microalgae. After 7-10 days, larvae use their foot to explore settlement sites (Cole, 1938) (Figure 1.4). Upon finding suitable substrate, often other oyster shells, they secrete a cement from their "cement gland" to attach permanently and begin benthic life (Bayne, 1969; Cole, 1938). This larviparous strategy, involving internal fertilization and brooding of a limited number of larvae, is probably well adapted to stable environments where parental protection enhances offspring survival, whereas *Crassostrea*'s high-output broadcast spawning, producing vast numbers of free-dispersing larvae, may provide greater resilience under variable and disturbed conditions (Gray et al., 2022).

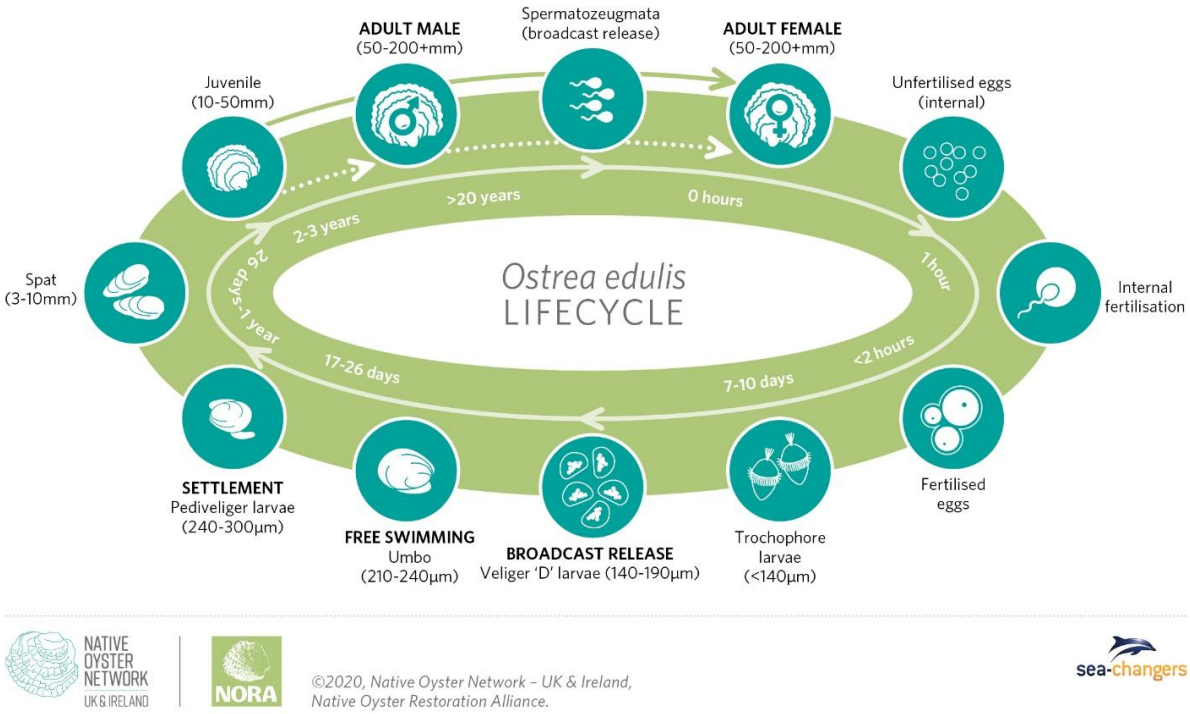


Figure 1.4. Life cycle of *Ostrea edulis*, adapted from Helmer et al (2019). ©2020, Native Oyster Network - UK & Ireland, Native Oyster Restoration Alliance (NORA).

1.1.3 History of the flat oyster and aquaculture

Understanding the historical trajectory of *Ostrea edulis* is essential to anticipate its future and inform conservation and aquaculture strategies. Oysters have been part of the human diet since prehistoric times and have been commercially exploited for over two millennia (Yonge, 1960). In Europe, *O. edulis* was widely consumed from Roman times onward, forming the basis of European oyster fisheries up until the late 19th century (Gosling, 2003).

From the 18th to 19th centuries, the increased use of destructive fishing practices such as dredging and bottom trawling led to severe overexploitation, habitat degradation, and a dramatic decline in natural oyster beds (Gosling, 2003; Orton, 1937; Pogoda, 2019; Pouvreau et al., 2023; zu Ermgassen et al., 2025). Möbius, 1877 described this depletion as "underwater deforestation", highlighting the scale of ecological impact. Historical reports indicate that *O. edulis* was once extremely abundant, forming extensive subtidal reefs and supporting vibrant coastal economies across Europe (Bennema et al., 2020; Olsen, 1883).

In response to the collapse of native oyster stocks, large-scale introductions of the Portuguese oyster (*Crassostrea angulata*) were carried out in the 19th and early 20th centuries. This species rapidly expanded, with annual production reaching up to 70,000 tons, before suffering a sharp decline in the 1960s due to viral disease outbreaks (Figure 1.5, example of the well documented French production to illustrate the European trend). These introductions unintentionally introduced pathogens and competitors that further accelerated the decline of *O. edulis* (Gosling, 2003; Pouvreau et al., 2023).

Following the collapse of *C. angulata*, the Pacific oyster (*C. gigas*), imported from Japan, was introduced to Europe. Its adaptability and disease resistance led to its rapid dominance in oyster aquaculture. In France alone, production quickly rose to over 100,000 tons annually (Buestel et al., 2009), largely displacing *O. edulis* from many traditional farming areas (Figure 1.5).

In parallel, the 20th century also marked the emergence of scientific research into *O. edulis* cultivation. Early studies led to advancements in microalgae culture to feed larvae and improved hatchery production of spat (Bruce et al., 1940; Cole, 1937). By the 1960s,

standardized hatchery techniques for bivalve larvae had been developed (Loosanoff & Davis, 1963), laying the groundwork for modern aquaculture.

However, technological progress alone was not enough to reverse the species' decline. In the 1920s, *O. edulis* populations were weakened by what was likely the first recorded viral disease. Although partial recovery occurred, *C. gigas* became the preferred species for aquaculture due to its greater yield and resilience. Ultimately, two major parasitic diseases, *Marteilia refringens* and *Bonamia ostreae*, devastated remaining *O. edulis* stocks from the 1970s onwards (Buestel et al., 2009). These parasites affected both farmed and wild populations, eradicating the last significant natural beds in many regions.

Despite efforts including restocking, hatchery improvements, and stricter management, the recovery of *O. edulis* has remained limited. As of 2002, global aquaculture production of *O. edulis* represented less than 0.2% of total oyster production, with Spain (4,565 tons) and France (1,600 tons) as the primary producers (FAO, 2024). By 2016, this had further declined to below 2,000 tons globally. Wild oyster beds have followed a similar trend, with only fragmented and isolated populations remaining in parts of Europe (Bonham & Roberts, 2018).

According to the statistical office of the European Union (Eurostat), *O. edulis* made up only 3% of Ireland's total oyster production in 2020, with *C. gigas* accounting for 97%. This reflects the broader European context, where *O. edulis* persists mainly in small-scale restoration or niche farming initiatives, such as in Germany, Sweden, Norway, and Scotland, though overall production remains marginal.

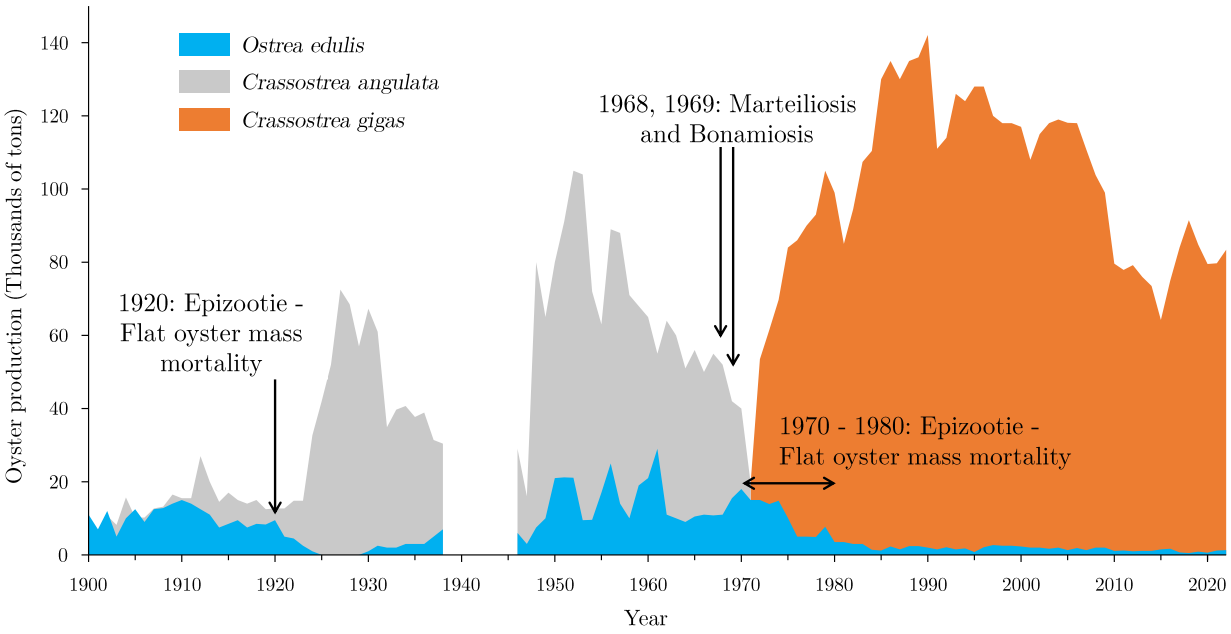


Figure 1.5. Evolution of the production of the three species of oysters farmed in France. Example of the French oyster production trend (in thousands of tons) since the beginning of oyster farming in relation to various historical milestones, including the emergence of various viral, bacterial and parasitic diseases. The blue area represents *Ostrea edulis* trend, the grey area the *Crassostrea angulata* production and the orange area the *Crassostrea gigas* production. Original illustration based on historical production data (Buestel et al., 2009) and official aquaculture data published since 2012 by the Ministry of Agriculture (https://www.agreste.agriculture.gouv.fr).

1.1.4 Focus on the German Bight and Helgoland

Historically, the native European flat oyster had a wide distribution in the German Bight, including the North and East Frisian Wadden Sea, the Helgoland Oyster Bed, and the offshore North Sea Oyster Ground, located 100-150 nautical miles from shore at depths of around 40 meters. This offshore area alone spanned an impressive 20,000-25,000 km² (Berghahn & Ruth, 2005; Pogoda, 2019) (Figure 1.6A).

One notable site, the Helgoland Oyster Bed, was situated approximately 4 nautical miles east-southeast of Helgoland, on the north side of the Helgoland Deep Rinne (54°09'00.0"N, 8°00'00.0"E), at a depth of 23-28 meters and covering an area of just 0.8

km² (Möbius, 1877). Although small compared to the 50 oyster beds up to 2 km long once documented by Möbius, 1877 in the Schleswig-Holstein intertidal flats (Germany), the Helgoland bed was of considerable economic importance. Harvesting was traditionally carried out using sailing cutters equipped with iron dredges (Figure 1.6B). Catch statistics from the main fishing period (1873-1886) show remarkably high yields relative to the bed's size. For example, 200,000 oysters were harvested over just 15 days in the 1873/1874 season, and in 1877/1878, catches reached 500,000 oysters over 21 days (Boos et al., 2004; Caspers, 1950). Following a sharp decline in yields in 1878, fishing was suspended from 1879 to 1882. However, intense exploitation resumed immediately afterward, with 170,000 oysters harvested during only 9 days in the 1882/1883 season.
Although no detailed records exist of the reef-like biogenic structures these oyster beds may have formed, the high yields suggest that dense oyster aggregations likely covered the seafloor. As early as the 17th century, overfishing was already recognized as a persistent problem for oyster stocks in the region (Gercken & Schmidt, 2014). The Helgoland Oyster Bed, in particular, declined rapidly. By 1900, the Biological Institute of Helgoland (BAH) estimated the remaining population at approximately 1.5 million individuals, predominantly aged between 20 and 30 years, indicating poor recruitment and a lack of juveniles (Boos et al., 2004). Environmental stressors, including cold winters and infestation by the boring sponge *Cliona ciliate*, further weakened the population.
During the mid-19th century, newly discovered offshore oyster grounds in the open North Sea were subjected to intensified exploitation by Dutch and German fisheries. These operations increasingly relied on industrial methods such as paddle steamers pulling up to six dredges simultaneously (Figure 1.6C). Möbius, 1877 observed that trawls during peak harvest months (August-October) could yield up to 1,000 oysters per capture. Similar patterns of collapse followed in the North and East Frisian Wadden Sea, where both oysters and the rich biogenic habitats they formed disappeared entirely.
By the 1920s, commercial oyster fishing in Germany had ceased due to economic inviability (Hagmeier & Kändler, 1927). Since the 1950s, *O. edulis* is considered functionally extinct along the German coast. Declines and local extinctions have also occurred across the broader region; for example, the species was presumed extinct in the

Dutch Wadden Sea by 1940, though small remnant populations have been rediscovered in recent decades (De Bruyne et al., 2013).

These historical oyster beds supported remarkably rich ecological communities. Caspers, 1950 documented the biodiversity of the Helgoland Oyster Bank, and Möbius, 1877, impressed by the complexity of oyster-associated life, introduced the now-fundamental ecological concept of biocenosis (or "living species community") based on these beds.

In recent years, *O. edulis* has been nominated for inclusion into the OSPAR (Oslo-Paris Commission) List of Threatened and/or Declining Species and Habitats and has been identified as a priority for conservation within OSPAR Region II: the Greater North Sea, Northeast Atlantic (OSPAR, 2010).

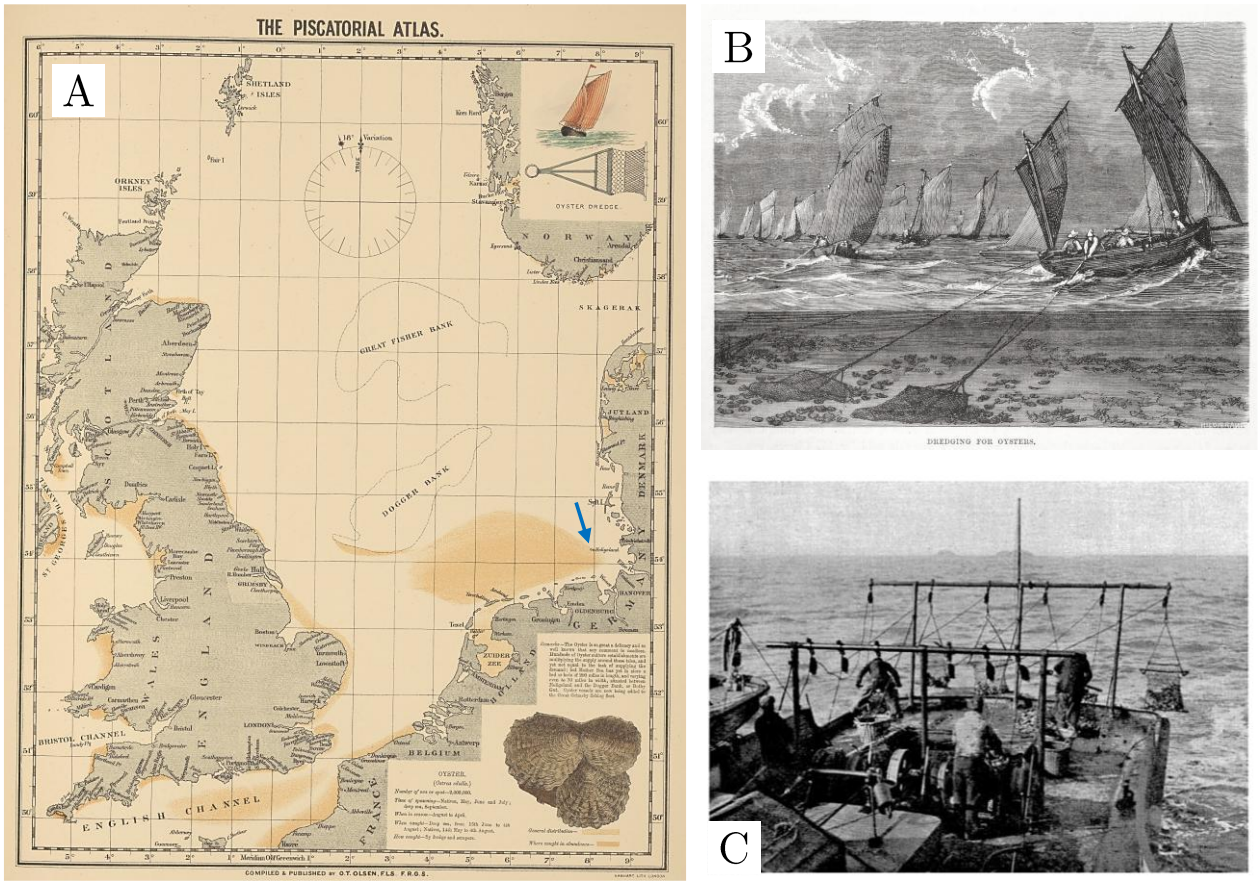


Figure 1.6. Historical distribution and exploitation of the European oyster in the North Sea. (A) Piscatorial AtlasMap on the distribution of *O. edulis* in the North Sea (Olsen, 1883). The island of Helgoland is indicated by a blue arrow to the east of the oyster bank. (B) Illustration of the oyster dredging (from Whymper, 1884, p. 229). (C)

Six dredges simultaneously used for oyster harvesting in the mid-19th century around Helgoland (from Hagmeier & Kändler, 1927).

1.2 Re-introduction of the species

1.2.1 Recent re-introduction efforts

In recent years, marine habitat restoration has gained momentum across Europe, driven by growing awareness of the degradation of vital ecosystems such as seagrasses, salt marshes, kelp forests, and biogenic reefs such as shellfish reef habitats. Among these, the native European flat oyster has emerged as a priority for restoration, due to its ecological importance and historical collapse (Airoldi & Beck, 2007; zu Ermgassen et al., 2025). Once widespread along European coasts, *O. edulis* has suffered dramatic declines due to centuries of overfishing, habitat loss, disease, and competition from non-native species (Airoldi et al., 2008; Pouvreau et al., 2023; Thurstan et al., 2023). Recognizing its role as a keystone species and reef-builder, the species and the habitat are listed as threatened and/or declining by the Oslo-Paris-Commission under the Convention for the Protection and Conservation of the North-East Atlantic (OSPAR, 2009). Biogenic reefs are protected under EU Flora-Fauna-Habitat Directive (FFH Directive, 92/43/EWG) (Council of the European Union, 1992) and the Marine Strategy Framework Directive (MSFD) (Council of the European Union, 2008). Since 2012, several countries, such as France, Germany, Ireland, the UK, the Netherlands, and Sweden, have launched initiatives to reintroduce and restore this species. In support of these efforts, the Native Oyster Restoration Alliance (NORA), formed in 2018, has played a key role in connecting practitioners, scientists, and policymakers across Europe. NORA emphasizes the need for coordinated, science-based restoration to establish self-sustaining oyster populations that contribute to biodiversity, water quality, and ecosystem resilience (Pogoda, Boudry, et al., 2020; Pogoda et al., 2019a; Preston et al., 2020). Initiative and programs across Europe are now actively deploying hatchery-produced juveniles and habitat structures in target sites. In the German Bight, a restoration program has also been ongoing since 2016 to sustainably reintroduce the European oyster in the Natura2000 area Borkum Reefground, with a breeding facility and underwater

experimental site on Helgoland. The project aims to develop and implement the oyster's ecological function in a historically relevant reef environment (Merk et al., 2020; Pogoda et al., 2023). While Europe still lags behind more advanced programs in the United States and Australia, the recent surge in collaboration and technical capacity marks a turning point. The native oyster is no longer just a historical relic, it is becoming a central figure in Europe's marine restoration narrative, offering both ecological benefits and a renewed connection to coastal heritage.

1.2.2 Oyster reef re-introduction benefits

Restoring native oyster reefs offers wide-ranging ecological benefits that extend far beyond the species itself. As ecosystem engineers, *O. edulis* creates complex three-dimensional reef structures that increase habitat heterogeneity, support high taxonomic diversity, and enhance ecosystem functioning through multiple trophic interactions (Waltham et al., 2020; zu Ermgassen et al., 2016; zu Ermgassen et al., 2020). First, these reefs create structurally complex environments that can stabilize sediments and buffer coastal erosion (Figure 1.7) (Preston et al., 2020; Thomas et al., 2022). Then, they serve as nurseries and refuges for juvenile fish and invertebrates, increasing biodiversity and secondary production (Haelters & Kerckhof, 2009). The oysters' shells also provide hard substrate for sessile organisms, offering a critical surface for epibiota that is largely absent in soft-bottom marine environments. Research indicates that *O. edulis* reefs support an increasing diversity of associated species, highlighting their role as both a biogenic habitat and a keystone species in intertidal and subtidal ecosystems (Figure 1.7) (D. Smyth & Roberts, 2010). In addition to their biodiversity value, native oysters provide important ecosystem services: they improve water clarity and quality by filtering large volumes of water, with an average of 0.5 litres per hour and 0.1 litres per hour of water filtered by large and small adult oysters, respectively. They promotes nutrient cycling, particularly denitrification in the sediments beneath reefs (Figure 1.7) (Fabra et al., 2024). These functions help regulate eutrophication and support broader coastal resilience. With the native oyster now largely absent from much of its historic range, restoration is increasingly seen not only as a conservation measure but also as a means of rebuilding lost ecosystem functions and services (Pogoda et al., 2019a; Preston et al.,

2020; zu Ermgassen et al., 2021). Restoration projects also create spill-over effects, where protected oyster beds seed surrounding areas, potentially supporting sustainable fisheries. The cumulative ecological and socio-economic benefits have led to strong support across Europe, recognizing oyster reefs as key targets for marine habitat restoration and long-term ecosystem recovery (BfN, 2020a, 2020b; Pogoda et al., 2019a; Preston et al., 2020; zu Ermgassen et al., 2021).

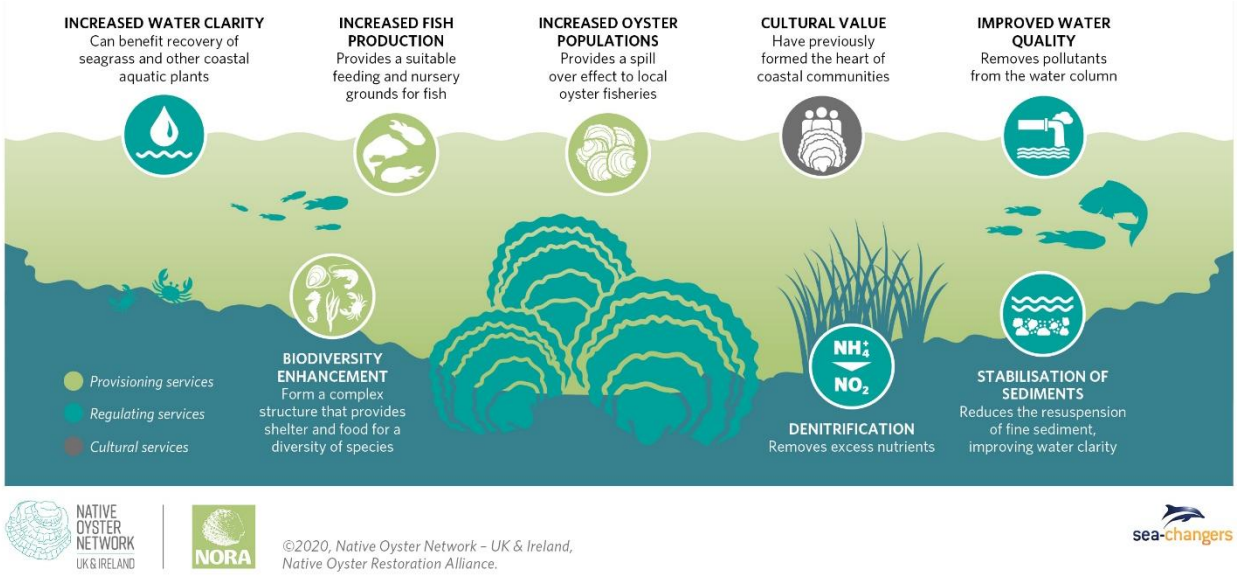


Figure 1.7. Ecosystem services provided by *Ostrea edulis*. ©2020, Native Oyster Network - UK & Ireland, Native Oyster Restoration Alliance (NORA).

1.3 Biological rhythms

1.3.1 Definition

Biological rhythms are recurring variations in physiological or behavioral functions that follow a predictable temporal pattern. These rhythms can range in duration from seconds to years and are observed across all levels of biological organization, from molecular processes and cellular activity to individual behavior and ecosystem dynamics (Aschoff, 1981). At the core of these rhythms lies an internal timekeeping mechanism, commonly referred to as a biological or molecular clock, which regulates the timing of biological processes. This internal clock is not fixed; it is synchronized by external environmental

cues known as *zeitgebers* (German term for "time-givers"). In the absence of these cues, the rhythm persists but follows its intrinsic, or free-running, period (Bell-Pedersen et al., 2005; Cermakian & Sassone-Corsi, 2000; Dunlap et al., 2004; Partch et al., 2014).
The evolution of life has been shaped by regular astronomical cycles, the rotation of the Earth on its axis (day-night), the orbit of the Moon around the Earth (lunar phases), and the orbit of the Earth around the Sun (seasons), which organisms have learned to use as reliable cues for timing key biological activities.(Andreatta & Tessmar-Raible, 2020; Helm et al., 2017). These rhythms are therefore adaptive, enhancing survival by enabling organisms to anticipate and prepare for predictable environmental changes (Yerushalmi & Green, 2009). As a result, a wide range of functions including feeding, locomotion, reproduction, and even cellular processes like gene expression and division can be governed by these environmental cycles. The diversity of biological rhythms reflects the complexity and specificity of life's synchronization to its environment, forming an essential link between organisms and their environment (Häfker et al., 2023; Helm et al., 2017; Mermet et al., 2017).

1.3.2 Short history

The concept of biological rhythms dates back to Antiquity, when naturalists such as Aristotle observed the seasonal timing of reproduction, flowering, hibernation, and migration. The first recorded description of a biological rhythm is attributed to Androsthenes in 325 BC, who noted the daily leaf movements of a tamarind tree on the island of Tylos (Bahrain) (Daan, 2010; De Mairan, 1729). For centuries, these rhythms were seen merely as responses to environmental cues.
A turning point came in 1729, when French astronomer Jean-Jacques d'Ortous de Mairan discovered that the heliotropic plant *Mimosa pudica* continued its daily leaf movements even in constant darkness, revealing the presence of an internal, autonomous rhythm, later termed "circadian." In the 18th century, Carl von Linné designed a "flower clock" based on the timed opening and closing of flowers (Linnaeus & Freer, 2003).
Progress accelerated in the 20th century. In 1935, German biologist Erwin Bünning showed that circadian rhythms in plants were genetically inherited, a major step in demonstrating their endogenous nature. In the 1950s, Jürgen Aschoff studied rhythms

in animals, including birds, and showed they too possess internal clocks. Around the same time, studies on honeybees revealed they could adjust their navigation dances based on the sun's movement, proving they could tell time. Similarly, Gustav Kramer found that migratory birds use solar cues corrected for time of day to orient themselves, confirming a sense of internal timekeeping (Kramer, 1950).

In 1954, Colin S. Pittendrigh demonstrated that circadian rhythms are stable across temperature ranges, introducing the idea of "*temperature compensation*", a hallmark of true biological clocks. He also proposed the existence of a universal biological clock mechanism across species (Pittendrigh, 1954).

Despite this progress, it wasn't until the 1960 Cold Spring Harbor Symposium, organized by Aschoff, Pittendrigh, Bünning, and Hastings, that chronobiology emerged as a unified scientific field. This meeting confirmed that biological rhythms are endogenous, genetically based, and a fundamental property of life (Aschoff, 1960; Daan, 2010; Pittendrigh, 1960). Two research approaches then developed: a systemic (macroscopic) view defended by Pittendrigh and Aschoff, and a molecular one, which would gain momentum in later decades.

A final landmark was reached in 2017, when the Nobel Prize in Physiology or Medicine was awarded to Jeffrey C. Hall, Michael Rosbash, and Michael W. Young for identifying the molecular mechanisms of circadian clocks in the fly *Drosophila melanogaster* (Bargiello & Young, 1984; Hardin et al., 1990). Their work firmly established that internal clocks are driven by genetic feedback loops, closing the loop between earlier behavioral observations and molecular biology.

1.3.3 Rhythm characteristics

Biological rhythms are periodic fluctuations that allow organisms to anticipate and respond to regular environmental changes, such as the daily light-dark cycle or seasonal variations (Emerson et al., 2008; Palmer, 2002; Yerushalmi & Green, 2009). They also ensure the temporal coordination of functions within cells, organs, organisms, populations, and ecosystems, helping maintain homeostasis and optimize energy expenditure (Boissin & Canguilhem, 1998; Moore-Ede, 1986).

Biological rhythms rely on three main components:

- An endogenous clock, present in every cell, based on a transcriptional/post-translational feedback loop (developed in the chapter 1.3.5);
- Environmental synchronizers, known as *zeitgebers*, such as light, temperature, or tides for instance (Sharma & Chandrashekaran, 2005);
- A functional rhythm, which translates clock signals into metabolic, physiological, and behavioral patterns.

In the absence of *zeitgebers*, the clock operates in free-running mode and reveals its intrinsic period, which is close to, but not exactly, the environmental cycle. This is why many rhythms are called "circa-" (meaning "about") rhythms. Importantly, these oscillations are stable but their characteristics vary between species and individuals (Aschoff, 1981).

A biological rhythm can be described by several parameters (Figure 1.8A):

- Period (τ): time between two identical events (e.g., two peaks in activity).
- Mesor: the average level of the rhythmic parameter over time.
- Acrophase: the time point of peak value.
- Bathyphase: the time point of lowest value.
- Amplitude: difference between the peak (or trough) and the mesor.
- Phase: position of a specific point in the rhythm relative to a reference, usually expressed in degrees or radians.

We distinguish biological rhythms that are often designated in relation to their period (Boissin & Canguilhem, 1998) (Figure 1.8B):

- Ultradian rhythms: period < 20 hours.
- Circadian rhythms: ~24 hours ($\pm$ 4 h), synchronized with the day-night cycle.
- Infradian rhythms: period > 28 hours.

Among these, four “circa-” rhythms are commonly recognized (Andreatta & Tessmar-Raible, 2020; Aschoff, 1981) (Figure 1.8B):

- Circatidal: ~12.4 h rhythms, linked to tidal cycle.
- Circadian: ~24 h rhythms, linked to the light-dark cycle.
- Circarsemilunar: ~14.8 days rhythms, linked to neap-spring tidal cycle.
- Circalunar: ~29.5 days rhythms, linked to lunar phases.

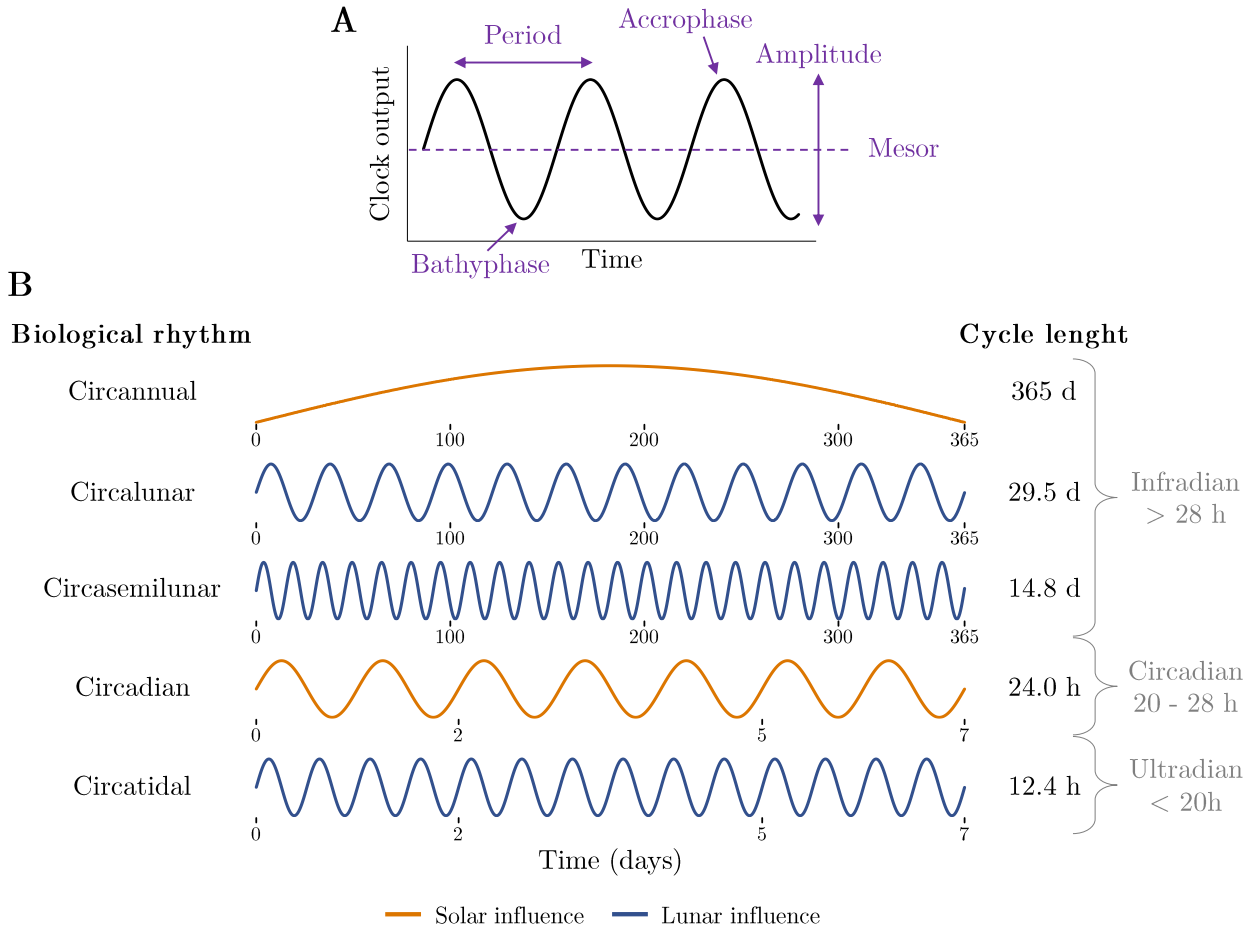


Figure 1.8. Parameters and categories of biological rhythms. (A) Schematic clock output oscillation illustrating rhythm parameters: period, mesor, amplitude, acrophase and bathyphase. (B) Representative oscillations of biological rhythms with different cycle lengths (circannual, circalunar, circasemilunar, circadian, circatidal), with period indicated on the right. Rhythms are positioned within ultradian, circadian, or infradian categories (grey). Biological rhythms influenced by solar cycles are shown in orange; those under lunar influence are in blue (for circatidal and circasemilunar, the color indicates the major influence).

1.3.4 The circadian rhythm

Circadian rhythms are endogenous biological rhythms with a period of approximately 24 hours, allowing organisms to anticipate and adapt to the daily light-dark cycle. These

rhythms are among the most extensively studied biological processes, particularly in well-established terrestrial model organisms such as plants, mice, and *Drosophila* (Dunlap, 1999). In mammals, the central circadian clock resides in the suprachiasmatic nuclei (SCN) of the brain, but circadian clocks also exist in peripheral tissues like the liver, heart, and even in non-nucleated cells like red blood cells (Dardente et al., 2007; Guo et al., 2006). Circadian rhythms can persist without environmental cues, but are normally synchronized by *zeitgebers*, such as light or temperature (Box et al., 1994). Across diverse taxa, including vertebrates (Zhang & Kay, 2010), insects (Dubruille & Emery, 2008; Hardin, 2005), and crustaceans (Zhang et al., 2013), the molecular architecture of circadian systems is broadly conserved, although specific components can differ among phyla. Circadian organization is generally associated with improved health, performance, and survival, while misalignment between internal time and environmental cycles typically reduces fitness (Bloch et al., 2013). Nonetheless, interspecific variation is considerable: some species exhibit weak or absent daily rhythms without apparent ill effects, suggesting ecological and evolutionary plasticity in the circadian system.

1.3.5 The circannual rhythm

Circannual rhythms are endogenous biological cycles with a period of roughly 365 days, enabling organisms to anticipate and adapt to predictable seasonal changes in photoperiod, temperature, and resource availability (Gwinner, 2012; Visser et al., 2010). These rhythms have been extensively documented in vertebrates, where they regulate processes such as migration, reproduction, and hibernation (Gwinner, 1989; Helm & Lincoln, 2017; Sáenz de Miera et al., 2014). Evidence from marine ectotherms, including crustaceans and bivalves, indicates that circannual rhythms also coordinate key life-history events such as growth, diapause, behavior, and seasonal metabolic adjustments (Baumgartner & Tarrant, 2017; Häfker et al., 2018; Payton, Sow, et al., 2017; Tran, Andrade, et al., 2020). Circannual rhythms can persist under constant conditions, suggesting a strong endogenous basis (Aschoff, 1955; Gwinner, 1981), and are entrainable by seasonal cues such as day length (Gwinner, 2012). After formalizing the concept of endogenous circadian rhythms, Aschoff (Aschoff, 1955, 1958) proposed extending these principles to annual cycles, highlighting hibernation, migration, and equatorial species

as particularly rewarding systems for the study of circannual rhythmicity. Building on this foundation, circadian clocks are now thought to contribute to circannual timing, either through hierarchical organization of oscillators or through cumulative circadian-driven signals that structure seasonal schedules. A clearer understanding of circannual rhythms is therefore essential to reveal how organisms integrate daily and seasonal temporal information to optimize survival, reproduction, and overall fitness.

1.3.6 The molecular clock

The modern era of molecular chronobiology began in 1971 when Konopka and Benzer identified the *Period* (*Per*) gene in *Drosophila*, after observing that mutations in this gene altered circadian rhythms, producing short, long, or arrhythmic phenotypes (Konopka & Benzer, 1971). In the 1990s, additional clock genes were discovered, progressively revealing the underlying molecular mechanisms. Homologs of these genes have been found in a wide range of organisms, from bacteria to vertebrates (e.g., humans, mice, zebrafish, frogs, and birds), highlighting the evolutionary conservation and ubiquity of the molecular clock (Dunlap, 1999). A unifying principle across species is the transcription–translation feedback loop (TTFL), a cellular oscillator that operates independently in each cell (Cermakian & Sassone-Corsi, 2000; Dunlap, 1999; Partch et al., 2014). It generates rhythmic activity through a negative feedback system, cycling roughly every 24 hours in the absence of external cues (Roenneberg & Merrow, 2005). In mammals, the core feedback loop involves the CLOCK and BMAL1 proteins, which activate the transcription of *Per* and *Cryptochrome* (*Cry*). The resulting PER and CRY proteins accumulate, form dimers, and inhibit CLOCK-BMAL1 activity, stopping their own transcription until protein levels drop and a new cycle starts (Figure 1.9A) (Shearman et al., 2000; Zhang & Kay, 2010). In *Drosophila*, a similar first loop exists: CLOCK (CLK)/CYCLE (CYC) (the BMAL1 homolog) activates *Per* and *Timeless* (*Tim)*, whose proteins then block CLK/CYC, generating daily rhythms (Figure 1.9B) (Glossop et al., 1999). *Drosophila* also has a second loop that controls *Clock* itself, here, CLK/CYC activates both a repressor (VRILLE) and an activator (PDP1ϵ) that act at different times to produce rhythmic *Clock* expression (Figure 1.9B) (Cyran et al., 2003). In mammals, *Bmal1* replaces *Clock* in this second loop, with REV-ERBα and ROR

serving as the repressor and activator, respectively (Figure 1.9A) (Jetten & Joo, 2006; Zhang & Kay, 2010). In both systems, these paired loops strengthen the clock's rhythms and help adapt them to environmental changes. These clock components also regulate numerous downstream "clock-controlled genes" (ccg) via E-box elements in their promoters, transmitting circadian signals to physiological pathways (Figure 1.9). Recently identified new regulators of the circadian clock, such as *Clockwork Orange* (*Cwo*) (Figure 1.9B), involved in the secondary feedback loop of *Drosophila* (Matsumoto et al., 2007) or *Doubletime* (*Dbt*) and *Shaggy*, which modulate clock protein stability through post-translational regulation (Figure 1.9B) (Nawathean & Rosbash, 2004). These factors help fine-tune the timing and robustness of rhythmic cycles. For instance, one such example is the clock-controlled temporal regulation of *hydroxyindole-O-methyltransferase* (*Hiomt*), which participates in melatonin synthesis, a hormone conserved across taxa and linked to daily, lunar, and seasonal rhythms (Foulkes et al., 1997; Reiter, 1993; Tosches et al., 2014). The presence of such diverse regulatory elements highlights the intricate, multilayered nature of the circadian machinery, particularly in marine organisms, where much remains to be uncovered.

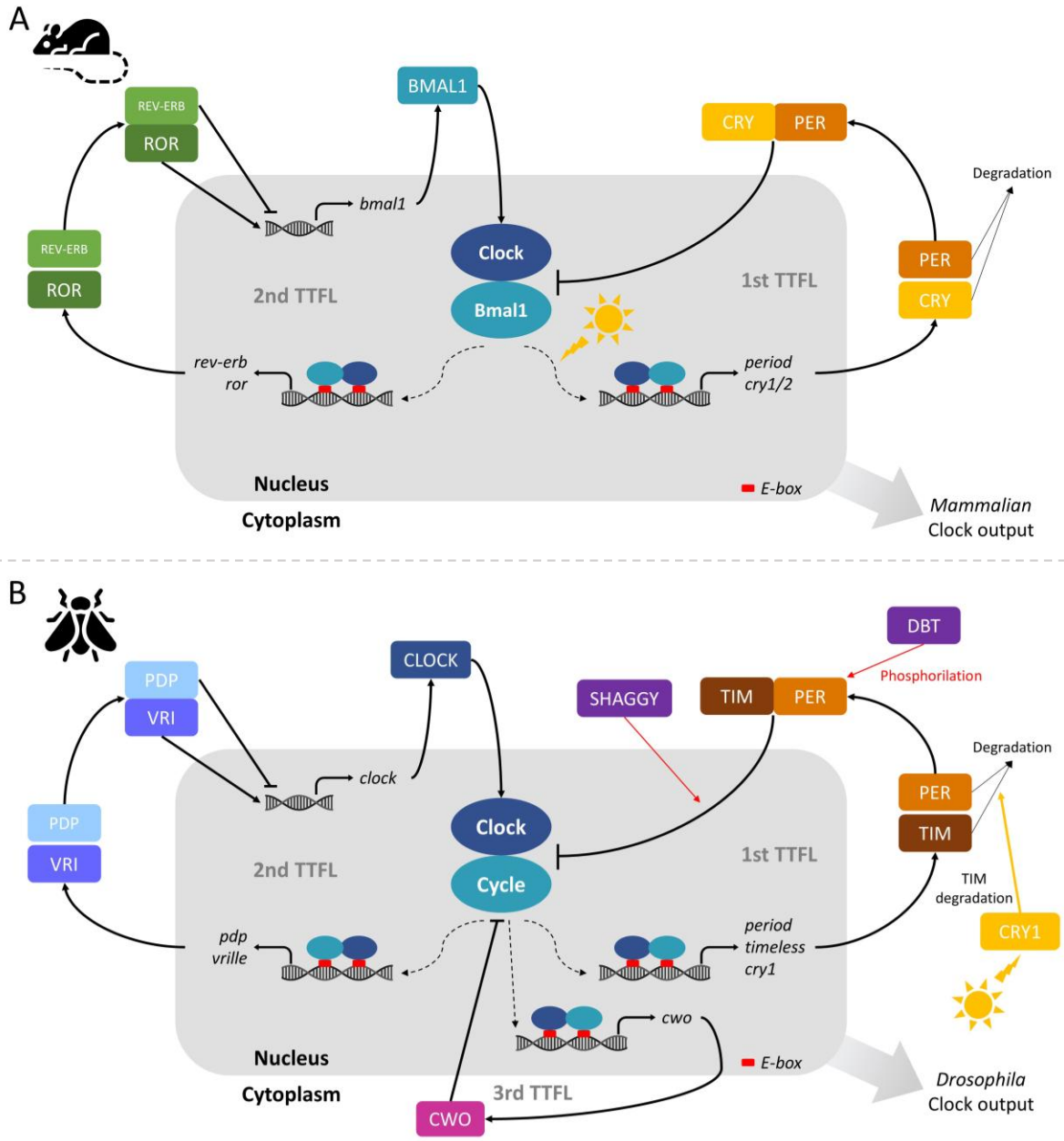


Figure 1.9. Schematic representation of transcriptional-translational feedback loops (TTFLs) underlying the circadian clock in mammals and *Drosophila*. (A) In mammals, the primary loop consists of CLOCK and BMAL1 heterodimers binding to E-box elements (shown in red) in the promoters of *Per* and *Cry* genes. PER and CRY proteins accumulate, form complexes, translocate into the nucleus, and inhibit CLOCK-BMAL1 activity. Phosphorylation by CK1 (δ/ε) and GSK3β regulates clock protein stability, nuclear translocation, and degradation. A secondary loop involves CLOCK-

BMAL1 activation of *Ror* and *Rev-erb* genes, whose products compete for ROR response elements in the Bmal1 promoter, thereby regulating *Bmal1* transcription. (B) In *Drosophila*, the primary loop involves CLOCK-CYCLE (CLK-CYC) dimers binding E-box elements (shown in red) to activate *Per* and *Tim* transcription. PER and TIM form complexes, enter the nucleus, and inhibit CLK-CYC activity. Phosphorylation by DOUBLETIME (DBT) and SHAGGY modulates protein function. Light activates CRYPTOCHROME (CRY), which targets TIM for degradation. A secondary loop consists of CLK-CYC activation of *Pdp1* and *Vri*, whose products compete in the *Clock* promoter. A third loop involves CLK-CYC regulation of *Cwo*, which in turn represses CLK–CYC activity. Inspired from Harper, 2017 and Rey, 2011.

1.4 Biological rhythms in marine organisms

Marine organisms have evolved within a highly dynamic environment shaped by multiple overlapping natural cycles. In addition to the daily light-dark cycle, they are also influenced by other periodicities such as tidal, lunar, and annual cycles (Naylor, 1999; Palmer, 1995; Tessmar-Raible et al., 2011). These environmental oscillations result in drastic and recurring changes, especially in coastal habitats, affecting salinity, temperature, light exposure, pressure, water currents, and food availability. Intertidal species, for instance, alternate between aquatic and aerial conditions with each tide. These periodicities influence their physiology, behavior, and reproduction. Despite the evolutionary significance of marine life, given that life is thought to have originated in the ocean, and the enormous biodiversity of marine ecosystems, chronobiological research has historically focused far more on terrestrial model organisms than on their marine counterparts.

1.4.1 The coastline ecosystem: A highly variable habitat

In marine environments, astronomical cycles strongly shape environmental conditions, especially along coastlines. These cycles arise from the gravitational and centrifugal forces exerted primarily by the Moon, due to its proximity, and by the Sun, due to its mass (Häfker et al., 2023; Tessmar-Raible et al., 2011).

Key astronomical rhythms include:

- Solar day (24 hours): One full rotation of the Earth relative to the Sun, driving the familiar day-night cycle.
- Lunar day (~24.8 hours): One full rotation of the Earth relative to the Moon, which causes the delay between successive high tides and produces the tidal rhythm (~12.4 hours) (Figure 1.10 A and B).
- Synodic lunar cycle (29.53 days): Time between two new moons (Figure 1.10D); this governs the lunar illumination cycle and drives the spring-neap tidal cycle (~14.76 days), which modulates tidal amplitude (Figure 1.10C).
- Anomalistic lunar cycle (27.55 days): Time between two lunar perigees (closest approach to Earth); this affects both the magnitude of spring tides and the intensity of moonlight (Figure 1.10E).
- Sidereal lunar cycle (27.32 days): Time it takes the Moon to complete one orbit around Earth relative to the stars, which causes latitudinal oscillations of tidal influence (Figure 1.10D).

In addition to lunar cycles, the annual revolution of the Earth around the Sun (365 days) generates the annual cycle, driving seasonal variation in photoperiod and temperature that strongly influences marine productivity and reproduction. Moreover, tidal dynamics are further shaped by non-solar tidal constituents (NSTC), such as diurnal and semidiurnal harmonics, which interact with lunar and solar forcing to create local variability in tidal amplitude and timing. These overlapping cycles contribute to the complex temporal landscape in which marine organisms live, imposing multifaceted rhythmic pressures that they must detect, anticipate, and adapt to for survival.

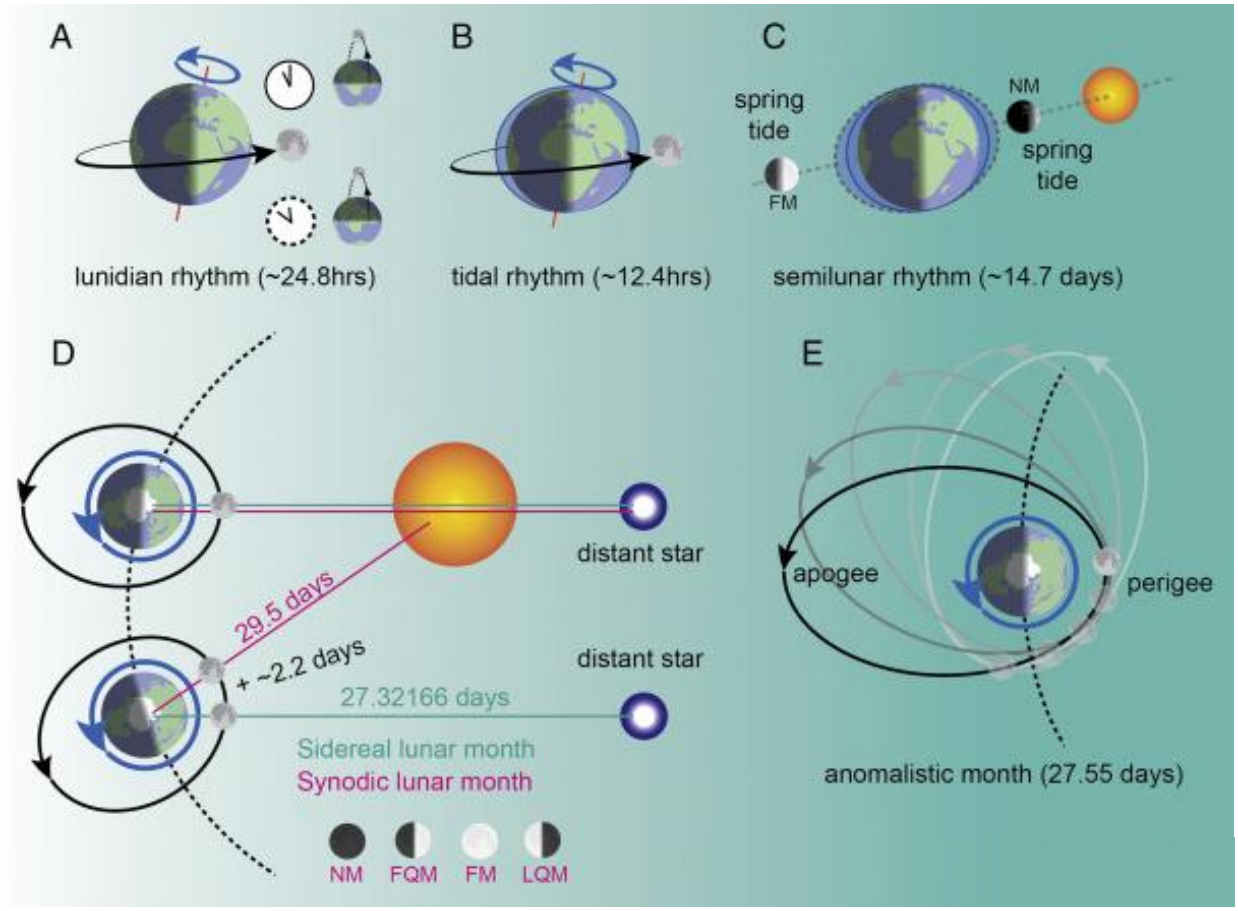


Figure 1.10. Schematized representations of the known astronomical and geophysical rhythms dictated by the Moon. (A) Lunidian rhythm, the period between two consecutive maximal heights of the Moon in the sky (meridian transits). (B) Tidal rhythm: water levels increase twice every day in two opposite sites on Earth, with one being the closest to our satellite. (C) Semilunar rhythm: the alignment of Moon, Earth and Sun potentiate tidal variations, creating spring (during the full moon, FM, and new moon, NM) and neap tides (during the first quarter moon, FQM, and last quarter moon, LQM). (D) Synodic and sidereal months: the synodic month produces the familiar lunar phases: NM, FQM, FM, and LQM (also referred to as the third quarter moon). (E) Anomalistic month. Modified from Andreatta & Tessmar-Raible, 2020.

1.4.2 Chronobiology in marine organisms

Marine environments are shaped by a complex interplay of environmental cycles, including diel (~24 h), tidal (~12.4 h), lunidian (~24.8 h), lunar (~29.5 days), semilunar (~14.8 days), and seasonal rhythms. These cycles profoundly influence physiological and behavioral rhythms of marine organisms (Andreatta & Tessmar-Raible, 2020; Häfker et al., 2023). Many of these rhythms are governed by endogenous biological clocks, which allow organisms to anticipate environmental changes and thus confer significant adaptive advantages (Bell-Pedersen et al., 2005; Emerson et al., 2008; Yerushalmi & Green, 2009). However, a key challenge remains in distinguishing between truly endogenous rhythms and direct responses to environmental stimuli. Among marine invertebrates, rhythms display remarkable diversity and plasticity, reflecting the complexity of coastal and oceanic environments. Anticipating tidal oscillations through circatidal clocks provides a major adaptive advantage (Rock et al., 2022). Based on behavioral studies, three main hypotheses have historically been proposed to explain the coexistence of circadian and tidal rhythms: (1) the presence of two distinct unimodal circadian and circatidal clocks (Naylor, 2010); (2) two circalunidian clocks (~24.8 h) coupled in antiphase (Palmer, 2000); and (3) a single oscillator governing both rhythms (Enright, 1976), the latter increasingly supported by evidence in littoral species (Tran, Perrigault, et al., 2020).
Empirical studies further illustrate this diversity of timing systems and rhythmic patterns among marine invertebrates: the intertidal isopod, *Eurydice pulchra* displays partial uncoupling of circadian and circatidal rhythms (Oliphant et al., 2025; Zhang et al., 2013); the annelid *Platynereis dumerilii* exhibits autonomous peripheral rhythms in chromatophore cells that adjust to sunlight and moonlight (Arboleda et al., 2019; Zurl et al., 2022); and Antarctic krill, *Euphausia superba* synchronize daily gene expression rhythms with diel vertical migration and metabolic cycles (Piccolin et al., 2020). Diel and tidal rhythmicity have also been reported in the amphipod *Parhyale hawaiensis* (Hunt, 2016; Ramos et al., 2019) and in the cnidarian *Nematostella vectensis*, where the dominance of circadian versus circatidal activity rhythms depends on light conditions (Hendricks et al., 2012; Oren et al., 2015). These examples highlight the remarkable adaptability of marine chronobiological systems across phyla and the importance of considering multiple overlapping cycles when studying marine chronobiology.

Among mollusks, bivalves exhibit a particularly wide range of biological rhythms, from annual to daily and tidal rhythms, often varying greatly between species. Recent technological advances, particularly high-frequency non-invasive valvometry (HFNI), have enabled continuous, long-term monitoring of valve behavior, opening new perspectives for chronobiological research. By tracking shell opening and closing in real time, this method has been applied in both laboratory and natural settings to uncover temporal patterns of physiology and behavior (Andrade et al., 2016). For instance, the mussel *Mytilus spp.* exhibit strong annual cycles of growth and rhythmic activity correlated with photoperiod, while the scallop *Chlamys islandica* shows seasonal growth without consistent rhythmic valve movements (Tran, Andrade, et al., 2020). In C. gigas, long-term *in situ* monitoring revealed robust tidal and daily rhythms, likely controlled by a common oscillator (Mat et al., 2012; Tran, Perrigault, et al., 2020). These rhythms are modulated by an annual cycle that influences both their intensity and the daily activity pattern, which shifts from nocturnal in autumn and winter to diurnal in spring and summer (Mat et al., 2012; Payton, Sow, et al., 2017). Beyond circadian and circatidal rhythms, oysters also exhibit annual, synodic (lunar), anomalistic, and semilunar rhythms (Payton & Tran, 2019; Tran et al., 2011). Reproduction is coordinated by the interaction of multiple rhythms, including annual, anomalistic, lunar, semilunar, daily, and tidal cycles, highlighting the complex temporal regulation of spawning in this species (Bernard et al., 2016; Payton, Sow, et al., 2017). Together, these studies illustrate how C. gigas provides a rich model for exploring the diversity and plasticity of biological rhythms in marine invertebrates. Similarly, the fan mussel *Pinna nobilis* displays both daily and lunar gaping rhythms, linked to sunset and lunar illumination (Garcia-March et al., 2016). In the European flat oyster, preliminary field and laboratory studies have revealed tidal-related valve gape cycles (Tonk et al., 2023). While these studies used valve activity primarily as a proxy for feeding behavior and were limited by small sample sizes, they still provide valuable insights and underscore the need for more comprehensive investigations. Together, such chronobiological approaches enhance our understanding of the temporal organization of marine bivalves and offer valuable tools for ecological monitoring under changing environmental conditions.

1.4.3 Impacts of global environmental change on marine rhythmicity

Global environmental change, including ocean warming, acidification, and deoxygenation, is exerting increasing pressure on marine habitats, with growing concern over its effects on the temporal organization of marine life (Brierley & Kingsford, 2009; Häfker et al., 2023). While much attention has been given to physiological and ecological responses, the potential disruption of endogenous biological rhythms remains underexplored. Light pollution, for instance, have been shown to desynchronize mass spawning events in reef-building corals, which rely on a precise alignment of diel, lunar, and seasonal cues (Ayalon et al., 2021). Similarly, earlier sea ice breakup in polar regions is shifting the timing of phytoplankton blooms and has been connected to declines in the stocks of key species such as the krill *E. superba* (Atkinson et al., 2019). Ocean acidification may influence reproductive timing and success by altering environmental cues and chemical signaling involved in gamete release, as observed in corals, marine worms, and fish (Baghel & Pati, 2015; Liberman et al., 2021; Watson et al., 2003). Meanwhile, the expansion of oxygen minimum zones is driving latitudinal and vertical distribution shifts, exposing organisms to novel light and temperature regimes, which could interfere with circadian and photoperiodic entrainment.

In addition to climate-related drivers, novel anthropogenic pressures are emerging as significant disruptors of biological timing. Artificial light at night (ALAN) is increasingly altering natural photic regimes in coastal ecosystems. In 2010, 22.2% of the world's coastlines (excluding Antarctica) were exposed to nightly artificial light pollution (Davies et al., 2014; Davies & Smyth, 2018; Falchi et al., 2016). Sky brightness is increasing by nearly 10% annually (Kyba et al., 2023). Known and potential impacts of ALAN on marine life include suppression of zooplankton diel vertical migration, disruption of settlement and spawning in invertebrates, aggregation of fish under pier lights, and disorientation of sea turtles (Davies et al., 2014). Beyond these broader ecosystem-level effects, ALAN has also been shown to disrupt circadian and seasonal rhythms in a range of marine species, including bivalves such as *C. gigas* (Botté, Payton, & Tran, 2023a, 2023b). Similarly, noise pollution from shipping, offshore construction, and other industrial activities introduces chronic low-frequency vibrations into marine habitats. Such acoustic disturbances can elicit strong behavioral responses in bivalves

such as *C. gigas* and *Mytilus edulis* (Charifi et al., 2017; Roberts et al., 2015) and may act as artificial *zeitgebers* or, conversely, as sources of desynchronization, with potential long-term consequences for fitness and population dynamics (Peng et al., 2015; Tidau & Briffa, 2016).

Together, these pressures highlight how organisms that have evolved finely tuned endogenous clocks over evolutionary timescales are now facing unprecedented environmental instability. While endogenous rhythms allow organisms to anticipate and prepare for predictable cyclic changes, the rapid pace and multifactorial nature of anthropogenic change threaten to induce mismatches between internal timing mechanisms and external cycles. Understanding the sensitivity and resilience of biological clocks in marine organisms is therefore critical for predicting ecosystem responses under ongoing global change.

1.5 Research objectives of the thesis

In this context, the aim of my thesis was to fill critical gaps in the chronobiological knowledge of *O. edulis*, with the main objective of improving our understanding of its resilience to environmental change and supporting ongoing protection and restoration efforts. To this end, the project combines molecular and behavioral approaches in the laboratory at the Arcachon Marine Station (France) and in the field on the island of Helgoland (Germany). In the laboratory, I focus on characterizing the endogenous clockwork of *O. edulis* and its regulation of key physiological processes, while in the field I investigate how these rhythms interact with natural environmental cycles (Figure 1.11). The behavioral component primarily focuses on valve activity and shell growth, which serve as functional indicators of feeding dynamics, energy allocation, and overall condition. In addition, monitoring these traits can also provide valuable insights into spawning behavior, offering a window into reproductive timing that is crucial for understanding population renewal. Together, this integrative framework aims to establish the first chronobiological foundation for *O. edulis*, linking endogenous regulation to environmental drivers across biological scales.

This project is structured into four complementary work packages (Figure 1.11):

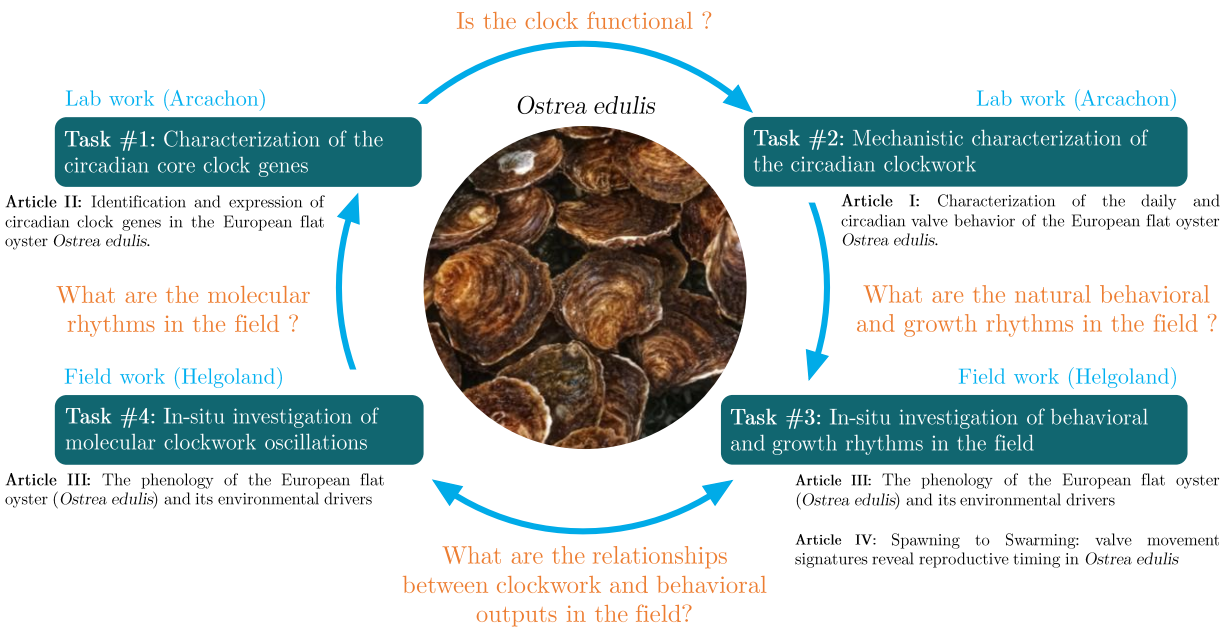


Figure 1.11. Overview of the "Flat Oyster Clock" project. The four main research tasks (dark blue squares) are shown together with their corresponding guiding questions (in orange). Publications associated with each task are indicated alongside.

1.6 Scopes and structure of the dissertation

1.6.1 Article I (Task #2) - Characterization of the daily and circadian valve behavior

Task #2 is central to our understanding of the oyster's intrinsic timekeeping mechanisms. Using non-invasive valvometry, this article (Le Moal, Tran, et al., 2025) allowed to successfully characterized the daily behavioral rhythms of *O. edulis* under controlled laboratory conditions (Figure 1.12). Our findings provide strong evidence for the presence of a functional endogenous circadian clock in this species, as demonstrated by the persistence of a diel rhythm under free-running conditions and its ability to be synchronized by environmental light cues. Importantly, the observed plasticity of this rhythm indicates an adaptable clock system capable of adjusting to environmental changes, while also highlighting the oyster's vulnerability to disturbances such as light

pollution. This article establishes a crucial foundation for subsequent *in situ* investigations by confirming the existence and nature of the internal clock mechanisms. Characterizing the biological clock of *O. edulis* provides a crucial first step toward understanding its rhythmic organization. This work not only reveals how an internal clocks shape key behavioral patterns but also establishes the foundation for interpreting field observations, thereby informing assessments of oyster resilience and guiding conservation strategies in the context of climate change and habitat restoration.

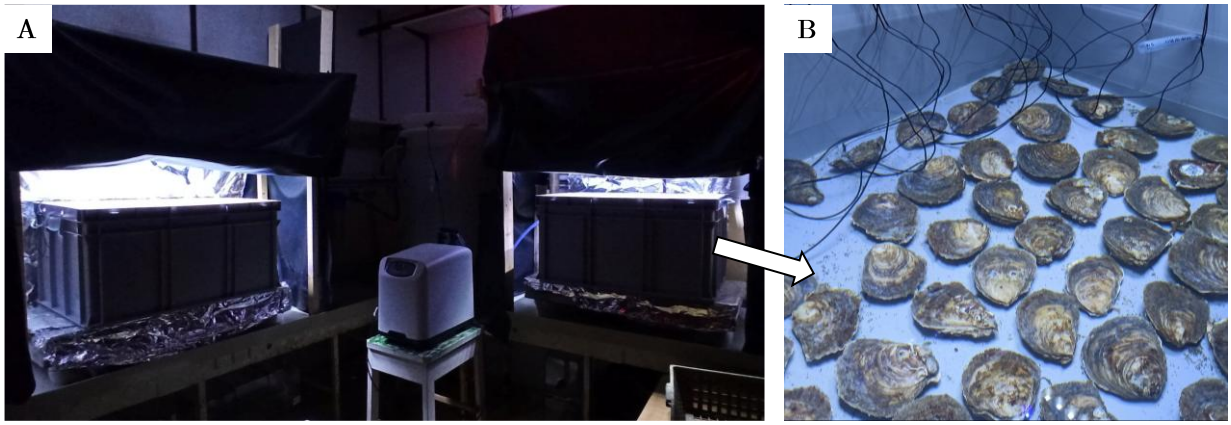


Figure 1.12. Laboratory experiment setup. (A) Two experimental units (EU) containing oysters exposed to controlled light regimes. (B) Oysters for dissection and individuals equipped with electrodes for continuous valve activity monitoring.

1.6.2 Article II (Task #1) – Identification and expression of circadian clock genes

Task #1 aims to identify and characterize the core circadian clock genes in *O. edulis*. This article (Le Moal *et al.*, 2025, under review) provides the first molecular characterization of the circadian machinery in *O. edulis*. Through phylogenetic and protein domain analyses, I successfully identified orthologs of core clock genes as well as clock-associated regulators, genes linked to melatonin synthesis, and photoreceptive pathways. This work further revealed tissue-specific rhythmic expression of several genes under controlled light regimes, confirming the presence of an active and plastic endogenous clock also at the molecular level. Importantly, these findings establish the

essential genetic framework that underpins circadian regulation in this species and demonstrate its evolutionary conservation across taxa, while highlighting unique features of the oyster's timing system. By connecting gene-level dynamics to the observed rhythms in valve behavior, this article provides a molecular foundation for subsequent field investigations, where both behavioral and molecular rhythms can be assessed under natural environmental cycles. Characterizing the molecular clockwork of *O. edulis* is therefore a critical step toward understanding how this species synchronizes with its environment, and ultimately toward predicting its resilience under ongoing climate-driven change.

1.6.3 Article III (Task #3 and Task #4) - Phenology of *Ostrea edulis* and its environmental drivers

Task #3 and Task #4 focus on investigating the phenology of *O. edulis in situ* and identifying the environmental drivers shaping it. This article (Le Moal *et al.*, 2025, submitted) provides the first long-term, integrative analysis of valve activity, shell growth, and clock gene expression of the flat oyster under natural conditions (Helgoland, German Bight). By combining high-resolution valvometry (Figure 1.13) with environmental monitoring and molecular profiling, I reveal that *O. edulis* exhibits coordinated daily, tidal, lunar, semilunar, and annual rhythms. These findings demonstrate how endogenous regulation interacts with natural environmental cycles to shape oyster phenology, while also highlighting the species' sensitivity to anthropogenic stressors. Importantly, this study establishes valve behavior and shell growth as functional biomarkers of environmental synchronization, offering new tools for monitoring population health. By linking molecular, physiological, and behavioral scales in the field, this work advances our understanding of *O. edulis* resilience and informs conservation and restoration strategies for this endangered ecosystem engineer.

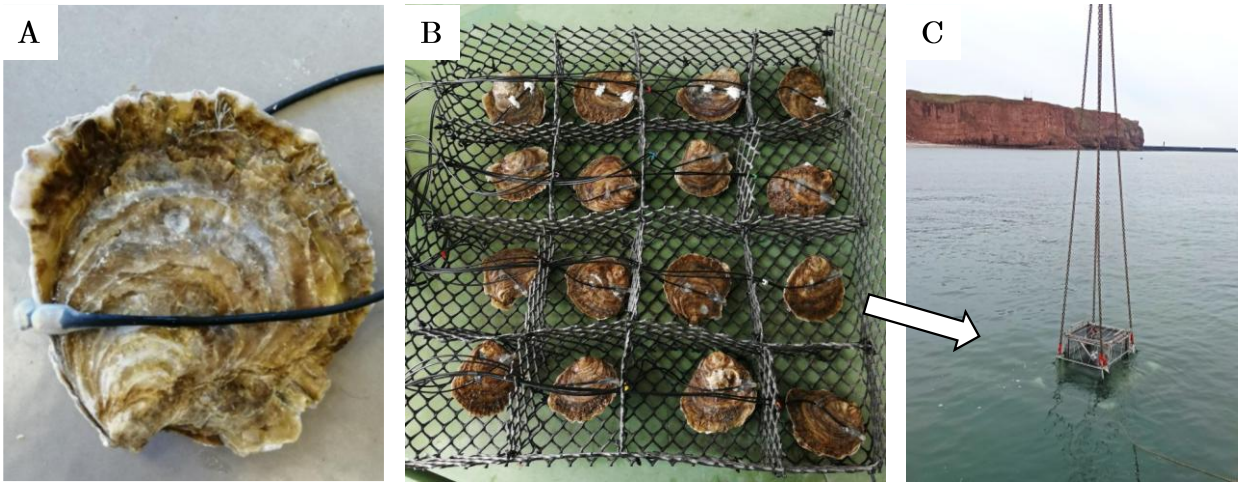


Figure 1.13. Field experiment setup. (A) Oyster equipped with a pair of lightweight electrodes to monitor valve activity. (B) Basket containing 16 oysters, each placed in individual chambers for continuous monitoring. (C) Deployment of the lander in Helgoland (German Bight), containing the high frequency non-invasive (HFNI) valvometer, the oyster basket with equipped individuals and environmental biosensors.

1.6.4 Article IV (Task #3) – *Ostrea edulis* valve behavior during reproduction events

In Task #3, we extended our chronobiological framework to the reproductive scale, investigating the fine-scale dynamics of *O. edulis* directly in the field. This article (Le Moal *et al.*, in prep.) presents the first continuous, *in situ* description of sex-specific spawning, fertilization, incubation, and larval release (larval swarming) behaviors using high-frequency non-invasive valvometry. Over two consecutive spawning seasons, we characterized the complete reproductive sequence and its synchronization with environmental cycles, while also documenting rapid sex changes within individuals. These findings not only validate and refine century-old observations but also reveal new mechanisms of reproductive plasticity. By providing a modern, high-resolution framework of *O. edulis* reproduction, this work delivers essential insights for the conservation and restoration of this endangered reef builder.

2. List of Manuscripts

Manuscript I - Characterization of the daily and circadian valve behavior of the European flat oyster *Ostrea edulis*

Alexandre LE MOAL, Damien TRAN, Laura PAYTON, Bernadette POGODA, Bettina MEYER

Published (29 April 2025, https://doi.org/10.1038/s41598-025-98746-x) - Scientific reports

Study Design and Methodology, all authors; Experiment, A.LM., D.T. and L.P.; Data Treatment, A.LM.; Interpretation, A.LM., D.T., L.P., B.P. and B.M.; Original Draft Writing, A.LM.; Manuscript Review and Editing, all authors; Funding acquisition, B.M., D.T., L.P. All authors contributed critically to the drafts and gave final approval for publication.

Manuscript II - Identification and expression of circadian clock genes in the European flat oyster *Ostrea edulis*

Alexandre LE MOAL, Laura PAYTON, Damien TRAN, Flore DARAMY, Audrey BOTTÉ, Bernadette POGODA, Bettina MEYER

Published (17 December 2025, https://doi.org/10.1098/rsos.250970) - Royal Society Open Science

Study Design and Methodology, all authors; Experiment, A.LM., D.T., L.P. and A.B.; Molecular work for Gills part, A.LM. and L.P.; Molecular work for Labial palps part, F.D., A.LM. and L.P.; Data Treatment, A.LM.; Interpretation, A.LM., D.T., L.P., B.P. and B.M.; Original Draft Writing, A.LM.; Manuscript Review and Editing, all authors; Funding acquisition, B.M., B.P., D.T., L.P. All authors contributed critically to the drafts and gave final approval for publication.

Manuscript III - The phenology of the European flat oyster (*Ostrea edulis*) and its environmental drivers

Alexandre LE MOAL, Damien TRAN, Laura PAYTON, Yannick GEEREBAERT, Bernadette POGODA, Bettina MEYER

Published (27 July 2026, https://doi.org/10.1038/s41598-026-63105-x) - Scientific reports

Study Design and Methodology, all authors; Experiment, all authors; Data Treatment, A.LM.; Interpretation, A.LM., D.T., L.P., B.P. and B.M.; Original Draft Writing, A.LM.; Manuscript Review and Editing, all authors; Funding acquisition, B.M., D.T., L.P., B.P. All authors contributed critically to the drafts and gave final approval for publication.

Manuscript IV - Spawning to Swarming: valve movement signatures reveal reproductive timing in *Ostrea edulis*

Alexandre LE MOAL, Damien TRAN, Laura PAYTON, Stéphane POUVREAU, Yannick GEEREBAERT, Bernadette POGODA, Bérenger COLSOUL, Bettina MEYER

Submitted in Nature communications

Study Design and Methodology, A.LM., D.T., L.P., S.P., Y.G., B.P. and B.M.; Experiment, A.LM., D.T., L.P., Y.G., B.P. and B.M.; Data Treatment, A.LM.; Interpretation, A.LM., D.T., L.P., S.P., B.P., B.C. and B.M.; Original Draft Writing, A.LM.; Manuscript Review and Editing, all authors; Funding acquisition, B.M., D.T., L.P., B.P. All authors contributed critically to the drafts and gave final approval for publication.

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3. Article I - Characterization of the daily and circadian valve behavior

Characterization of the daily and circadian valve behavior of the European flat oyster *Ostrea edulis*

Alexandre LE MOAL, Damien TRAN, Laura PAYTON, Bernadette POGODA, Bettina MEYER

Published in Scientific reports - https://doi.org/10.1038/s41598-025-98746-x

Abstract

Marine organisms exhibit a multitude of biological rhythms synchronized with the interactions of the Sun-, Earth-, and Moon cycles. However, the biological rhythms in bivalves remain poorly studied. This study focuses on the native European flat oyster (*Ostrea edulis*), an endangered species of coastal ecosystems and a key organism in restoring of biogenic reef habitats. We aim to determine whether a molecular endogenous circadian rhythm exists in *O. edulis* and to characterize its daily expression. To address these questions, the oysters' valve behavior, as an output of the circadian clock expression, was recorded under different light conditions and free-running regimes using non-invasive valvometry. This work demonstrates the existence of a circadian clock mechanism that generates a labile behavioral circadian oscillation under free-running conditions. In light:dark conditions, a diel rhythm appears nocturnal, synchronizable to a shift of light phase, and remains unmodified whether the oysters are fed or not. This rhythm anticipates light:dark changes, indicating its endogenous origin. Finally, when exposed to artificial light at night the daily behavior is disrupted. This study characterizes the circadian behavioral rhythm of *O. edulis*'s as plastic and labile. This plasticity would be advantageous in terms of ecological adaptability but increases sensitivity to anthropogenic pressures such as light pollution.

Keywords: *Ostrea edulis*, oysters, daily rhythm, circadian rhythm, valve behavior, free-running.

Introduction

The native European oyster *Ostrea edulis* (Linnaeus 1758) is a filter feeder formerly abundant in Europe from the North Sea, along the Atlantic coast and other European coastal waters including the Mediterranean and the Black Sea (Thurstan et al., 2023). *O. edulis* is a biogenic reef builder that plays a key ecological role and provides many ecosystem services (e.g. substrate formation and biodiversity enhancement) (Haelters & Kerckhof, 2009). Over the 20th century, stocks of *O. edulis* have been severely depleted by overfishing and additional anthropogenic stressors, such as invasive diseases and is now one of the most threatened marine habitats in Europe (Pogoda, 2019; Pouvreau et al., 2023). However, in recent years, conservation and active restoration of European oyster habitats across its former distribution range have become a major focus of ecological restoration efforts (Pogoda et al., 2019, 2020) to take advantage of the wide-ranging ecosystem functions and services this species and it reef habitats provide (Haelters & Kerckhof, 2009). Despite these recent efforts, knowledge of the general physiology of *O. edulis* has primarily centered on reproductive aspects. Notably, chronobiological studies of their behavioral and physiological traits are still lacking, which is crucial for understanding their ability to adapt to and fit within their cyclic and fluctuating environment.

Life on Earth involves exposure to cyclical environments that are related to the rotation of the Earth on its axis, the Earth's trajectory around the Sun, and the Moon's orbit around the Earth, as well as the interaction among these forces (Andreatta & Tessmar-Raible, 2020). To maintain harmony with their environment, organisms have evolved biological rhythms that enable anticipation and synchronization with their biotope (Tessmar-Raible et al., 2011). These biological rhythms are generated by an internal clock present in each cell of an organism (Bell-Pedersen et al., 2005). It consists of a self-sustained auto-regulatory network of transcriptional and translational feedback loops. These ancestral and endogenous clockworks are entrained by cyclic and reliable environmental factors called *zeitgebers* (time givers), such as light that synchronize the circadian clock producing daily rhythm (~ 24h) (Bell-Pedersen et al., 2005; Emerson et al., 2008; Yerushalmi & Green, 2009). This clock mechanism facilitates the temporal organization of many metabolic, physiological and behavioral functions of organisms (Helm et al., 2017; Mermet et al., 2017). In the absence of *zeitgebers*, the clock is termed «free-running» and expresses its endogenous period, which is close but not identical to the environmental cycle (Abhilash et al., 2017; Bertolini et al., 2019). By convention, rhythms with periods ranging from 20h to 28h are considered as circadian.

Studies on biological rhythms of marine organisms are increasing but they still lag behind those of their terrestrial congeners. Many of these behavioral rhythms are endogenous, controlled by molecular clock(s) and synchronized by cyclic environmental cues driven by solar and lunar light entrainments but also tides. In marine bivalves, studies of biological rhythms remain scarce. A good proxy for biological rhythms is the shell's movement, the valve activity behavior (Mat et al., 2012; Tran et al., 2016), which is closely related to respiration, nutrition, and reproduction. Previous *in situ* studies on bivalves have revealed that valve behavior could exhibit a multitude of rhythms such as tidal, daily, lunar and annual ones (Tran et al., 2011, 2020; García-March et al., 2008; Payton & Tran, 2019; Le Moal et al., 2023). Regarding the characterization of the clock mechanisms that underlie these rhythms, few studies have been conducted under controlled conditions, particularly on the Pacific oyster *Crassostrea gigas* (Mat et al., 2014; Mat et al., 2012) and the Blue mussel *Mytilus edulis* (Ameyaw-Akumfi & Naylor, 1987). Recently, a preliminary study has investigated the daily valve activity of *O. edulis* (Tonk et al., 2023). However, the characteristics of its rhythm and the underlying mechanism have not yet been described.

Anticipation of environmental cycles allowed by a clock mechanism provides an advantage for animal fitness (Krittika & Yadav, 2020). However, while endogenous clock systems improve species fitness under steady ecological conditions, a too robust clockwork could limit species' adaptation potential in a changing world due to global warming (Häfker et al., 2023). Furthermore, anthropic pressures, such as the recent threat of artificial light at night (ALAN) are increasing in all coastal ecosystems (Smyth et al., 2021). By masking natural variations of light, ALAN can disrupt the marine organisms' light perception and affect organisms' biological rhythms and, thus, their physiology. This disruption can have cascading effects on critical physiological processes such as feeding and reproduction, potentially leading to population declines (Davies et al., 2014; Gaston et al., 2017). This is consequently, in the context of re-introduction this endangered species, it is essential to investigate the chronobiology of the native European oyster *O. edulis* in its environment.

To achieve this goal, we first characterized the daily rhythm of valve activity using the high frequency non-invasive (HFNI) valvometer biosensor (Botté, Payton, & Tran, 2023a; Tran et al., 2023). We characterized this daily rhythm in terms of diurnal/nocturnal modality, robustness, synchronization by light/dark cycles, and response to food availability. Secondly, we investigated the existence of an underlying circadian clock mechanism responsible for this daily rhythm by studying valve behavior under free-running conditions and its anticipatory patterns under light/dark conditions.

Thirdly, we tested whether ALAN at low intensity (5 lx) could disrupt the circadian rhythm of *O. edulis*.

Material and methods

Experimental model and general conditions

All research detailed in this study complied with French law, and the experiment was conducted according to international ethical standards. The experiment took place in Arcachon, France (at the Marine Station, 44° 39′ 48″ N, 1° 9′ 49″ W) over a period of 97 days, from March to June 2023, involving 32 European native oysters *O. edulis* (74.7 ± 1 mm shell length and 72.5 ± 1 mm shell width, mean ± standard error (SE); approximately 8-10 years old). These European native oysters were collected at Loch Ryan in South West Scotland (54° 59′ 10″ N, 5° 03′ 18″ W) (Rossmore Oysters Ltd.) and acclimated for 4 months under natural light conditions in a continuous flow-through system using natural seawater from Arcachon Bay, supplement with a food supply of microalgae (*Chaetoceros calcitrans)*.

Experimental setup

Throughout the entire experiment, the oysters were isolated from external vibrations using an antivibration system and an isolated blind room to minimize any external influences on their valve behavior (Fig. 3.1A). Experiments were performed in two distinct experimental units (EU1 and EU2) (L × W × H:74.8 × 54.8 × 40.8 cm), containing approximately (~) 150 L of seawater, which was continuously supplied with filtered (< 1 µm) and oxygenated seawater at a flow rate of 350, maintaining a constant composition of 15 ± 0.1 °C, pH = 7.9 ± 0.1, salinity = 33.1 ± 0.1 ‰, mean ± SE. A 180 L retention tank was situated between the seawater supply and the two EUs to homogenize the seawater and prevent potential environmental cycle cues. The experimental setup and each EUs were surrounded by opaque black curtains to shield the experiment from external light contamination (Fig. 3.1A).

Experimental protocol

After 6 days of acclimatization in experimental design, eleven series (lasting 8–10 days) were conducted based on various photoperiods and other experimental cues (Fig. 3.1B). All times are presented in local time, i.e., UTC+1 (Coordinated Universal Time). The

11 series comprised: reference 12 L:12 D (8 d, photophase from 07:00 to 19:00 h, series 1, 3, 5, 7, 9, 11); D:D (8 d; series 2); 12 L:12 D with a 4h-phase shift (8 d, photophase from 11:00 to 23:00 h; series 4); dim L:L (8 d; series 6), 12 L:12 ALAN (8 d; photophase from 07:00 to 19:00 h; series 8), and 12 L:12 D with food supply (10 d, photophase from 07:00 to 19:00 h; series 10). Throughout all series the oysters were in unfed conditions, except for the series 10, where the oysters were continuously supplied with a solution of microalgae *Chaetoceros calcitrans* at a flowrate of 100 mL/min/EU to maintain a concentration of ~ 500 cell/mL in each EU.

Illuminances were measured underwater at the oysters' depth in lux (lx) in each EU using a handheld spectroradiometer (Blue-Wave UVN-100, StellarNet Inc.). Equivalent irradiance in µE/m²/s was also measured using a portable radiometer (MICRO Class, Profiling (in-water), E (irradiance) PAR sensor, Biospherical Instrument Inc.). During the photophase of all the 12 L:12 D series, the light intensity varied gradually to mimic the natural light cycle using programmable white (413–688 nm, peak at 551 nm) LED light bars (MH3SP3 DSunY). The maximum light intensity during daytime was 1406.1 ± 136.2 lx (mean ± SE) corresponding to 28.99 µE/m²/s in irradiance, occurring between 12:30 and 13:30 h, except for series 4, where the maximum of light intensity was between 16:30 and 17:30 h. During the scotophase, the light intensity was below the detection limit of the spectroradiometer (<0.05 lx). During the dim L:L condition (series 6), the light intensity was maintained continuously at 5.67 ± 0.34 lx (0.15 µE/m²/s), corresponding to a dim light exposure, to minimize masking effects. During the ALAN (series 8) condition of 12 L :12 ALAN, the oysters were exposed to 5 lx during the scotophase (Fig. 3.1B).

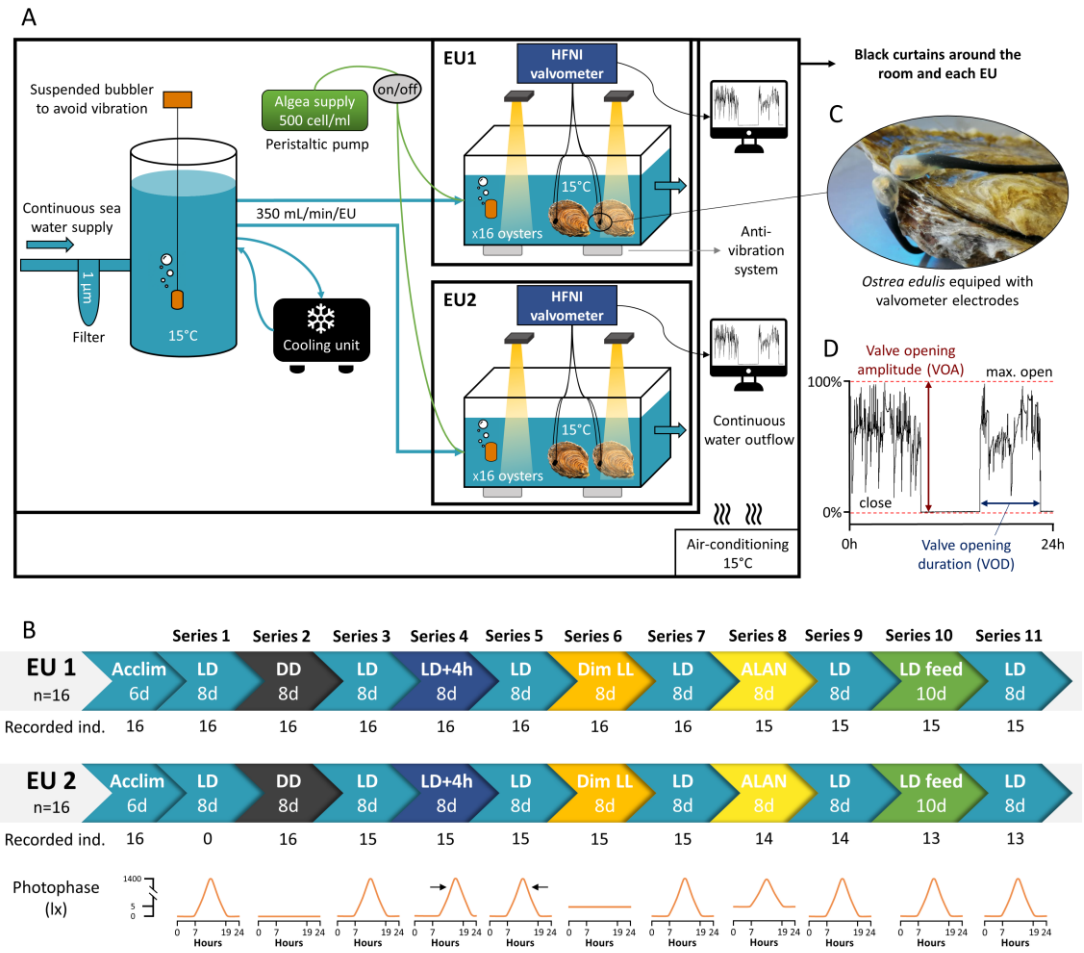


Figure 3.1. Experimental setup and protocol. (A) Experimental setup to investigate the circadian rhythm of *O. edulis*. (B) Experimental protocol. Timeline of the 11 series to which the oysters of both EUs are exposed successively. (C) Zoom of *O. edulis* equipped with HFNI valvometry electrodes for valve behavior recording. (D) Example of a daily individual valve activity showing the two behavioral parameters chosen in this experiment, i.e., the valve opening amplitude (VOA) and the valve opening duration (VOD). EU: experimental unit. HFNI: high frequency non-invasive.

Ostrea edulis valve behavior measurement and representation

The valve activity of 16 oysters per EU was measured using high-frequency non-invasive (HFNI) valvometers. They consist of a pair of lightweight electrodes (<100 mg) designed to minimize disturbance of the oyster behavior. The electrodes are glued on each half-shell and each electrode is linked to the HFNI valvometer by a flexible wire allowing undisturbed oyster valve movement (16 oyster monitored by valvometer) (Fig. 3.1C). An electromagnetic current is generated between the electrodes, allowing the

measurement of each oyster's valve activity every 4.8 s. Further details are available on (Tran et al., 2023) and (Le Moal et al., 2023). Raw daily recordings are processed using Labview 8.0 (National Instrument, Austin, TX, USA). It is important to note that for series 1, EU2 data are missing due to electrical failure (Fig. 3.1B).

To analyze the behavioral rhythm, two parameters of valve activity were measured: the valve opening duration (VOD) and the valve opening amplitude (VOA) (Fig. 3.1D). For each hour the individual VOD and VOA are measured. VOD is established from an opening threshold (5% of VOA) setting the state "open" or "closed" of the animal. If the oyster is continuously open for an hour, the percentage of hourly VOD is 100%. On contrary, if it does not open at all for an hour, the hourly VOD is 0%. Individual VOA of each oyster is reported each hour as a percentage where 100 % indicates that the valves opened at their maximum amplitude during the whole hour and 0 % indicates that the valves were closed during the whole hour, in between all the values of amplitudes are possible.

The mean hourly VOA and VOD are represented as double-plotted actograms and heatmaps, where each line represents two days (Fig. S9.1). In an actogram, hourly activity levels superior to the daily median are represented by dark blue (VOD) or red (VOA) bars, whereas hourly activity levels inferior to the daily median are represented by light grey bars. In heatmaps the mean hourly VOA and VOD are represented according to a color scale from 0 % to 100 %, divided into ten intermediate ranges.

Chronobiological analyses

Chronobiological analyses were performed using the software Time Series Analysis Serial Cosinor 6.3. Several steps were necessary to validate a significant rhythm. First, to assess the data quality, the absence of random distribution in the data set is checked using an autocorrelation diagram, and the lack of a stationary phenomenon is evaluated through a partial autocorrelation function calculation (Box et al., 1994). Next, the data were examined for periodicity ranging from 5 to 35 h using spectral analyses and the Lomb and Scargle periodogram (Scargle, 1982). This periodogram provides a significant threshold ($p = 0.95$). Finally, rhythmicity is validated by the Cosinor model employing the period identified by the Lomb and Scargle periodogram (Bingham et al., 1982). For a specified period, the model is expressed as $Y(t) = A\cos(\pi t/\tau + \varphi) + M + \varepsilon(t)$, where A represents amplitude, φ is the acrophase, τ is the period, M denotes the mesor and ε signifies the relative error. Two critical tests confirm the calculated model and the presence of a rhythm: the elliptic test must be rejected, and the probability for the null amplitude hypothesis must be < 0.05. A chronobiometric parameter was calculated: the

percent rhythm (PR, %) and the proportion of cyclic behavior explained by the model. For each series, binary VOA and VOD datasets represented in actograms were examined for rhythmicity. These analyses were conducted at the group level (EU1 + EU2, n = 32 individuals) and the individual level. Only rhythms within the range of 20 – 28 h were identified in this study. To address multiple testing in chronobiological analyses, a Benjamin-Hochberg $p < 0.05$ was considered significant. Raw results of individual analyses are available in Supplementary Table S9.1.

Results

Actograms and chronobiological analyses of valve activity

Figure 3.2 presents the actograms of the mean daily activity throughout the 97-day experiment, encompassing 11 series and the related results of chronobiological analyses based on individual daily activity illustrated in Figure S9.2. Two behavioral parameters were tested: valve opening amplitude (VOA, %) and valve opening duration (VOD, %). Under 12 L:12 D (series 1, 3, 5, 7, 9, 11), both VOA and VOD were synchronized by L:D exposure and exhibited a significant daily rhythm of ~ 24h. The robustness of the rhythm, measured as PR (%) ranged between 49.6 % and 63.4 % (PR = 56.6 ± 2.3 % mean ± SE) for VOA and between 41.0 % and 57.2 % (PR = 50.3 ± 2.3 % mean ± SE) for VOD. To assess the plasticity of the daily rhythm, the series 4 displays the VOA and VOD activities, following a + 4 h-light shift. The results indicate a maintained significant daily rhythm of 23.6 ± 0.1 h (PR = 44.4 %) for VOA and 23.8 ± 0.1 h (PR = 59.7 %) for VOD. To investigate the endogenous circadian rhythm, mean behavioral activity was assessed under two free-running conditions: D:D and dim L:L. Under D:D (series 2), *O. edulis* demonstrated a significant rhythm within the circadian range (20 – 28 h) for VOA (25.8 ± 0.1 h) and for VOD (26.4 ± 0.1 h). The associated PR values were 44.2 % for VOA and 40.7 % for VOD. Under dim L:L (series 6), no significant circadian rhythms were detected for either VOA or VOD. Under ALAN exposure (series 8), a notable daily rhythm of 23.6 ± 0.1 h was observed for VOA, with a decreased PR (PR = 41.5 %) in comparison to the L:D series. For VOD, no significant daily rhythm was noted. Although a significant periodicity (35.0 ± 0.1 h) was identified, using the Lomb & Scargle periodogram, the outcomes from the Cosinor model were not significant ($p = 0.139$). Finally, under feeding conditions (series 10), the results showed a significant daily rhythm of 23.9 ± 0,1 h (PR = 55.9 %) for VOA and 24.0 ± 0.1 h (PR = 57.3 %) for VOD.

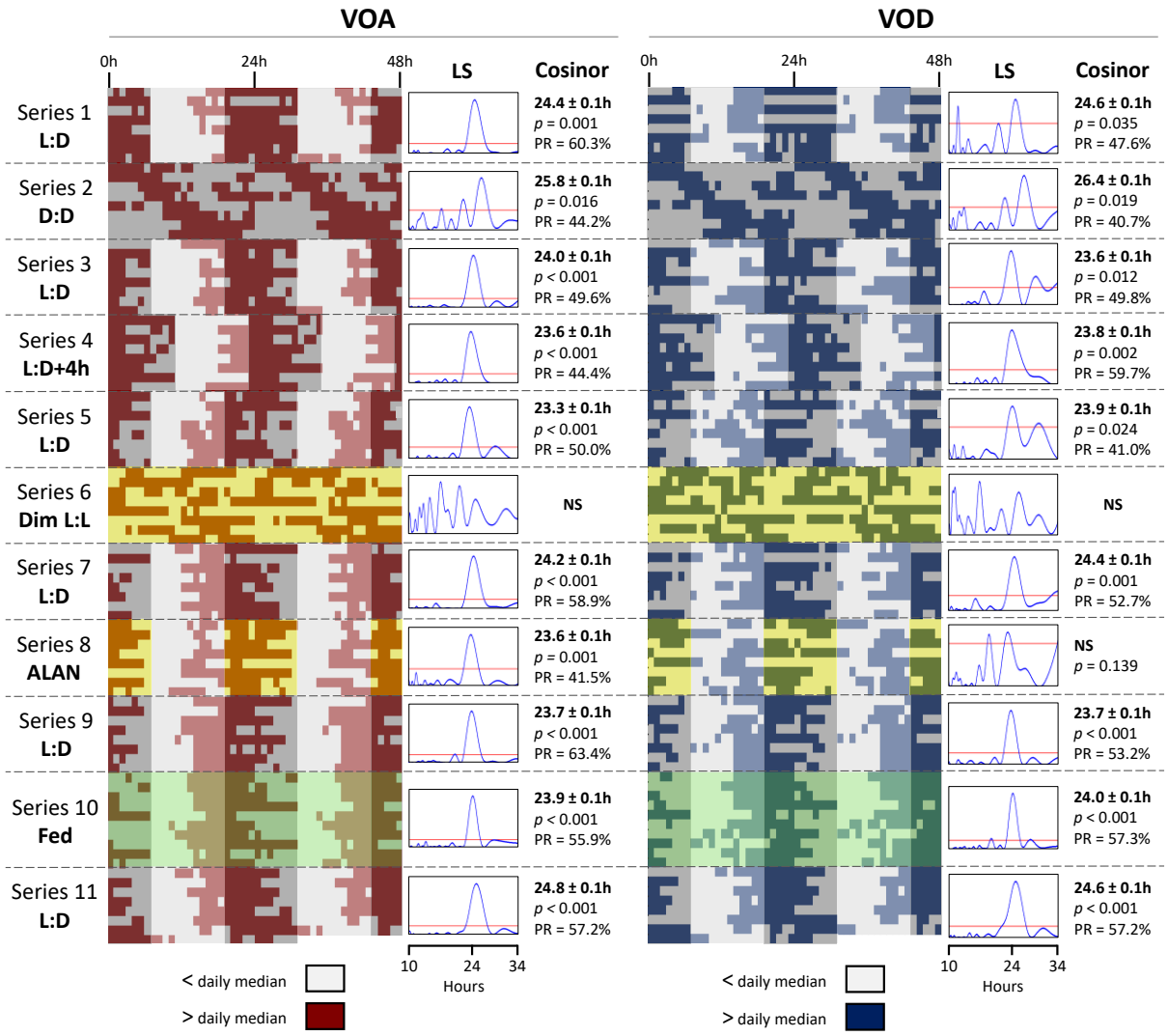


Figure 3.2. Actograms and chronobiological analyses of *O. edulis* valve activity. Actograms of mean VOA (left panel) and VOD (right panel) during the 11 series, 97 days in total (n = 16-32). Each actogram of each series is associated the Lomb & Scargle periodogram (LS) performed on binary data and characteristics of the Cosinor model: the period (h) ± SE, the *p-value* and the percent rhythm (PR, %). The red line on the LS periodogram indicates a threshold of significance ($p = 0.95$). The rhythm is considered significant for the Cosinor model with $p < 0.05$. On actograms, dark grey areas = scotophase, white areas = photophase, yellow area = dim light exposure, green area = continuous food supply. The 11 series are detailed in Fig. 3.1B.

Individual chronobiological analyses of valve activity

Figure 3.3 presents the results of individual chronobiological analyses of VOA and VOD across the 11 series, based on the data illustrated in Figure S9.2. Under 12 L:12 D (series 1, 3, 5, 7, 9, 11), the mean percentage of oysters exhibiting a significant daily rhythm was noticeably higher ($p < 0.001$, t-test) for VOA compared to VOD. For VOA, 52 ± 1 % of oysters demonstrated a daily rhythm, with a minimum of 48 % and a maximum of 58 %. In contrast, for VOD, 20 ± 4 % of oysters showed a daily rhythm, with a minimum of 6 % and a maximum of 38 %. In similar ranges, the percentages of rhythmic individuals during series 4 (L:D+4h), series 8 (ALAN), and series 10 (Fed) were 45 %, 52 % and 48 % for VOA, and 26 %, 17 % and 21 % for VOD, respectively. During the free-running D:D condition (series 2), the percentage of rhythmic oysters was only 6 % for VOA and 3 % for VOD (Fig. 3.3), attributed to prolonged closure events in a substantial number of individuals (Fig. S9.2). In the free-running dim L:L condition (series 6), no oysters exhibited a significant daily rhythm.

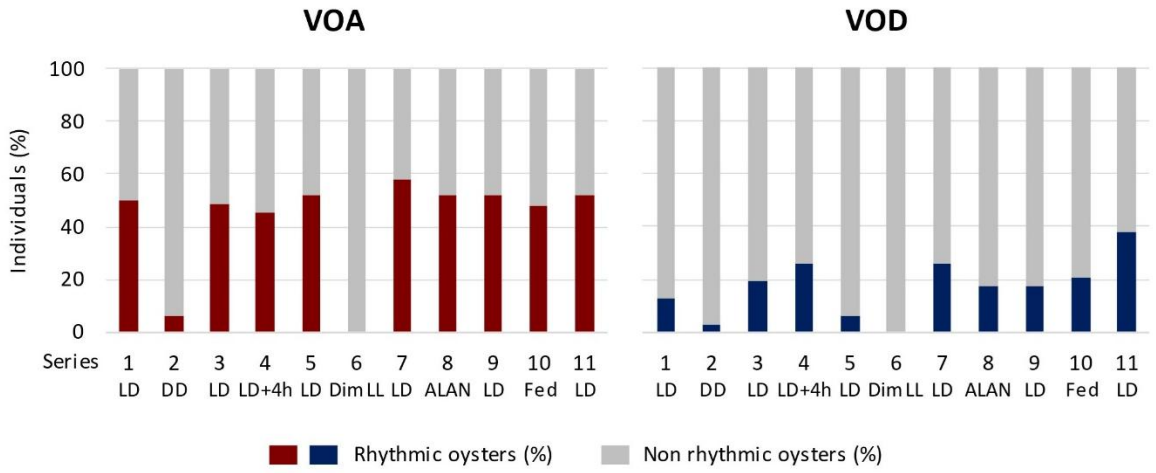


Figure 3.3. Individual chronobiological analyses of valve activity. Percentage of oysters with a significant daily rhythm for VOA (left panel) and VOD (right panel) during the 11 series (n = 16-32). The 11 series are detailed in Fig. 3.1B.

Daily pattern of valve behavior

Figure 3.4 illustrate the mean daily patterns of the VOA and VOD during the 97-day experiment corresponding to 11 series, based on the individual daily pattern shown on Figure S9.3. For each behavioral parameters the results are shown as heatmaps associated with the corresponding mean daily patterns based on the average of the days of each series. Under 12 L:12 D (series 1, 3, 5, 7, 9, 11), both VOA and VOD showed a minimal activity just after transition to light phase (20 – 25 % VOA and 35 – 45 % VOD) and increased during the whole photophase to reach a peak of activity at the

beginning of the night (40 – 60 % VOA and 55 – 65 % VOD). For all the series, VOA peak of activity was between 20 h and 22 h, i.e., 1 – 2 h after light off, while for VOD the peak of activity was between 19 h and 22 h, i.e., 0–2 h after light off. Individual daily VOA peak during L:D series are plotted in Figure S9.4 and showed a maximum of nocturnal peaks (75%). Under the + 4 h-light shift in series 4, both VOA and VOD nocturnal daily patterns were maintained and quickly synchronized by the shifted L:D regime, evidenced by the nocturnal peak of activity shifting by 4 h, i.e., still 1–2 h after light off. Under free-running conditions, mean daily VOA and VOD were significantly reduced in both D:D (series 2) and dim L:L (series 6). In D:D (series 2), the mean VOA peak of activity occurred at 0 – 1 h, reaching only ~ 16%, while the mean VOD peak was at 4 – 5 h, reaching only ~ 22%. In dim L:L (series 6), the mean VOA peak was also at 0 – 1 h, reaching only ~ 20%, whereas the mean VOD peak occurred at 5 – 6 h, reaching only ~ 28%. Under ALAN exposure (series 8), the peak activity of VOA was between 19 h and 20 h, i.e., 1–2 h earlier than the peak observed under the 12 L:12 D series, while the VOD peak occurred between 15 and 16 h, becoming diurnal, i.e., 4–7 h earlier than the peaks observed under 12 L:12 D. Under continuous feeding conditions, the VOA peak of activity remained between 20 h and 21 h, comparable to the peaks observed under the unfed 12 L:12 D series, while the VOD peak of activity was between 22 and 23 h, i.e., 1–3 h earlier than the peaks observed under the unfed 12 L:12 D series, and remained nocturnal. To complete and highlight individual nocturnal activity, the occurrence of daily peaks of VOA for the 32 individuals and the total for all individuals are detailed in Supplementary Figure S9.4.

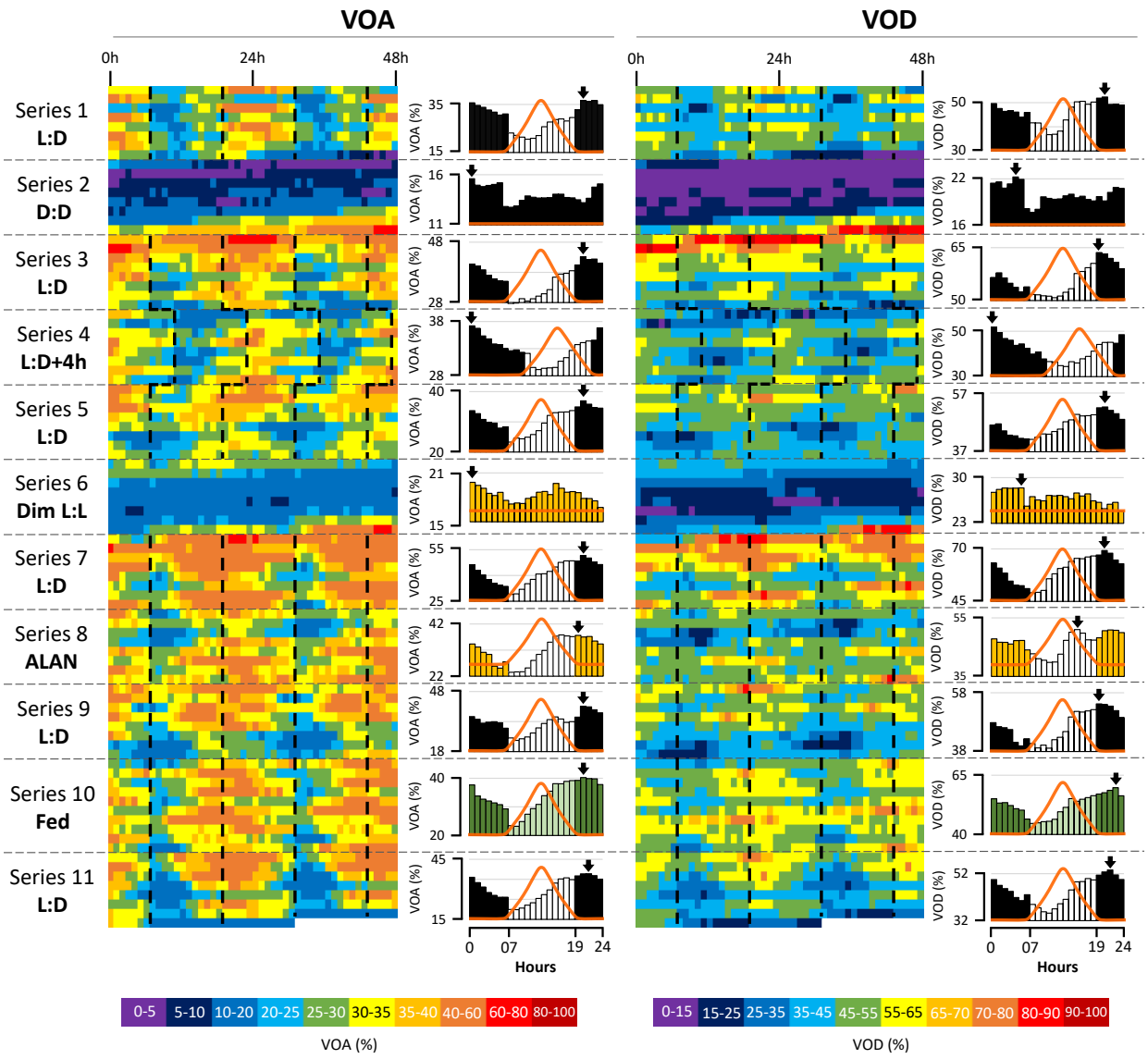


Figure 3.4. Daily pattern of *O. edulis* valve behavior. Heatmaps of mean VOA (left panel) and VOD (right panel) during the 11 series, 97 days in total (n = 16-32). For each heatmap of each series, a histogram of the corresponding mean daily pattern is associated. Black dotted line on the heatmaps separate scotophase and photophase. In histograms, dark bars = scotophase, white bars = photophase, yellow bars = dim light exposure, dark and light green bars = scotophase and photophase + feeding respectively. The black arrows show the daily peaks of activity. The orange lines represent the photoperiod for each series. The 11 series are detailed in Fig. 3.1B.

Anticipation of valve activity diel behavior to daily light cycles

To illustrate the anticipation of valve activity behavior in response to light cycle *zeitgebers*, Figure 3.5 presents the mean daily profiles of mean VOA and mean VOD for the six reference 12 L :12 D series (series 1, 3, 5, 7, 9, 11). For both parameters, no direct response to light (switch on or switch off) was noted. A gradual decrease in activity commenced during the night, reaching a minimum in the early morning, followed by a gradual increase of activity that peaked in the early night. No direct correlation was identified between light intensity and valve behavior. To emphasize that the rhythmic behavior is not a direct response to light, Supplementary Figure S9.5 displays the mean VOA and mean VOD profiles during the last day of series 1 (12 L :12 D) and the first two day of series 2 (D:D). The results indicate that during the second day of D:D, valve activity displays a similar rhythmic pattern to the L:D condition, despite a significant overall daily reduction in activity for both VOA and VOD.

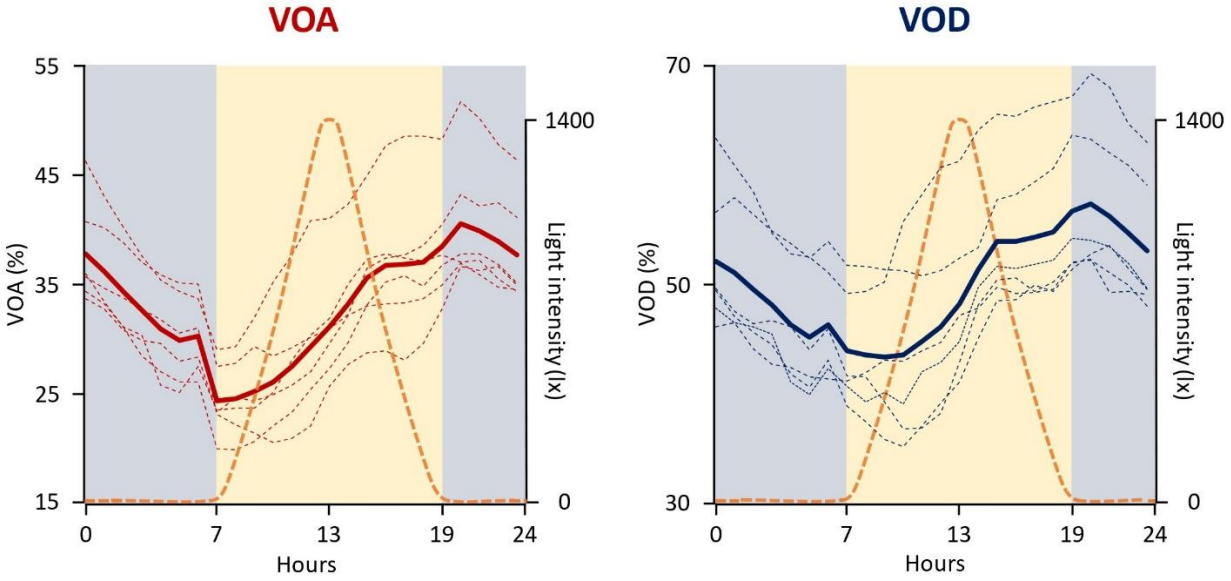


Figure 3.5. Anticipation of valve activity diel behavior to daily light cycles. Mean daily profile of mean VOA (left panel) and mean VOD (right panel) during the 6 references 12 L :12 D series (n = 16-32). In doted lines, the profiles of each series (n = 6 series, series 1, 3, 5, 7, 9, 11). In bold solid line, the mean profile of the 6 series. The dotted orange lines represent the photoperiod for the 6 references 12 L :12 D series.

Discussion

This study demonstrates a synchronized daily valve activity in *O. edulis* for both VOA and VOD parameters under L:D conditions, characterized by a nocturnal peak of activity. With food supply, only the VOD acrophase is slightly altered but remains nocturnal. At the individual level, VOA displays more robust significant rhythms than VOD. Under free-running conditions (D:D), group-level results reveal a circadian rhythm, indicating an underlying endogenous clock mechanism. This assumption is reinforced by the anticipation of the light phase observed in the valve activity pattern under L:D and maintained during D:D. *O. edulis'* circadian clock can be described as labile and plastic due to its ability to adapt rapidly to a shift in light regime. Furthermore, even though a significant rhythm is found under D:D at the group level, at the individual level, only ~ 6 % and ~ 3 % of oysters are rhythmic for VOA and VOD, respectively. Under dim L:L condition, no circadian rhythm was observed, suggesting a masking effect despite the low light intensity applied. Lastly, under ALAN condition, the VOD daily rhythm disappears, and the peak of activity becomes diurnal.

Characterization of O. edulis circadian clockwork.
Typically, rhythms in marine organisms are described as robust under entrainment but labile, weak, or noisy in constant conditions (Gwinner & Brandstatter, 2001; Last et al., 2009; Palmer, 1995), which is confirmed for the native European oyster *O. edulis*. Although the daily rhythm is noted to be strong under L:D conditions, labile rhythmic oscillations were observed in free-running conditions where the rhythmicity is noisy and dampened at the individual level, indicating the presence of a functional circadian clock but with weak inertia. Gwinner and Brandstätter (Gwinner & Brandstatter, 2001) also describe a labile circadian oscillator as having a capacity for rapid adaptation to *zeitgeber* phase shifts (Aschoff, 1981; Klotter, 1960). This observation is further validated for *O. edulis* as resynchronization to a phase shift in the L:D cycle was immediate, without transient cycles.
The results also indicated that *O. edulis* does not respond directly to the daily light cycle but anticipates it, further attesting to an endogenous mechanism that optimizes its fitness (Bloch et al., 2013; Dodd et al., 2005). Characterizing the robustness or the lability of the circadian clock could provide insights into the plasticity of the clock mechanism. A robust clock facilitates maximizing fitness in a stable cyclic environment. However, a clockwork that is too robust may be a limitation for the adaptation or expansion of the species, particularly for marine organisms. In coastal areas, numerous environmental cues can influence the behavior of resident species. Specifically, alongside

solar day cycles, marine organisms are subject to tidal cycles, which typically occur twice per lunar day (24.8 h), approximately every 12.4 h. It is now well documented, that marine organisms can exhibit a strong tidal rhythm in addition to the daily rhythm (Naylor, 2010; Tran et al., 2011; Webb, 1976). The phase shift of the lunar day by ~ 51 minutes each solar day also results in a permanent phase shift between the two rhythms. Although recent findings at the molecular level suggest an interplay of molecular mechanisms involved in different rhythms (Häfker et al., 2023), a fundamental debate continues regarding the existence and nature of one or several core clocks to elucidate both concomitant circadian and circa-tidal rhythmicity (Tran et al., 2020). Based on behavioral studies, three historical hypotheses have been proposed to explain the co-existence of tidal and daily rhythms. The first hypothesis, supported by Naylor (Naylor, 2010), posits the existence of two separate and unimodal circatidal and circadian clocks. The second hypothesis advocated by Palmer (Palmer, 2000), suggests the presence of two unimodal circalunidian clocks (24.8 h lunar day cycle) coupled in antiphase. Lastly, the third hypothesis originally advanced by Enright (Enright, 1976), proposes a single clock that governs both circadian and circatidal rhythms. This hypothesis has received strongly backing from findings at both behavioral and molecular levels concerning the oyster *C. gigas*(Tran et al., 2020) and has become increasingly accepted for various littoral species (Häfker et al., 2023). According to this hypothesis, a labile single clock would facilitate the adaptability to a phase shift between solar and lunar days and to modulate daily the bimodal pattern of behavior driven by both daily light:dark and tidal vibration cycles, the *zeitgebers* of this putative unique clock. Moreover, a labile/plastic unique clock would enable a marine species to adapt to the duration of tidal cycles that average 12.4 h, while not remaining equal to 12.4 h for each cycle due to modulation by local geographical (local biotope morphology and hydrodynamic) and meteorological (atmospheric pressure and wind) conditions. Thus, tides predominantly around 12.4 h, termed semi-diurnal tides, may, in certain parts of the world, oscillate at 24.8 h, referred to as diurnal tides, matching the lunar day period, and closely aligning with the daily period, necessitating considerable plasticity of the clockwork (Naylor, 2010). Similarly, the daily cycle, which exhibits less variability than that of the tidal one, predominantly remains around ~ 24 h, yet can vary significantly in terms of light phase duration, as observed in polar regions, where polar night (24 h of darkness) or polar day (24 h of light) can persist for months. In these areas, it has been demonstrated that the daily/circadian rhythms of bivalves are expressed, even during polar day or polar night, as seen in mussels *Mytilus sp.* (Le Moal et al., 2023; Tran et al., 2023) or in pecten *Chlamys islandica* (Tran et al., 2016).

What about a labile clock in an anthropized world exposed to global warming and the modification of the nocturnal lightscape due to ALAN?

Endogenous clocks are adaptive mechanisms finely tuned over evolutionary timescales. They enable organisms to anticipate predictable changes in their environment and therefore adjust their physiology and behavior in expectation of these changes. Thus, by preparing their physiological processes, organisms can optimize cyclic opportunities (e.g. food supply, appropriate temperature for specific physiological processes, etc.) and mitigate cyclic risks (e.g. predation, UV radiation etc.) inherent to their environment (Helm et al., 2017). However, the current rapid global climate change alters the temporal dynamics of biotic and abiotic environmental cycles to which endogenous clocks are adapted, while the daily light:dark cycle, i.e. the main *zeitgeber* for the circadian clock, is unaffected. Global warming could also induce latitudinal shifts in species distribution, as organisms track their optimal thermal environments toward the pole to avoid heat stress (Beaugrand et al., 2019; Bradshaw & Holzapfel, 2010; Helm et al., 2013; Kharouba & Wolkovich, 2020). As the duration of the light phase in the daily light:dark cycle is specific to each latitude and time, organisms' shift northward implies a new photic environment (Per Huffeldt, 2020). In both cases, whether there is a change in latitudinal or not, the proper alignment of endogenous rhythms and environmental cycles is challenged. The interpretation of the daily light signal by the endogenous clock must adapt to the new living environment to ensure the fitness of organisms. In this context, a labile clock, such as that described in *O. edulis*, may offer an adaptive advantage in coping with these rapid changes. Conversely, a clock that is too robust may increase the risk of a mismatch between organisms' biological rhythms and their new environment. Notably, temporal mismatches between species in a food chain can jeopardize the survival of organisms (Bradshaw & Holzapfel, 2010; Kharouba & Wolkovich, 2020; Per Huffeldt, 2020).

ALAN may also produce a risk of misalignment between endogenous rhythms and environmental cycles that disrupts the daily, seasonal, and lunar photic signals and hence could disrupt *zeitgebers* of endogenous clocks, constituting an unprecedented physical change over evolutionary time scales (Davies & Smyth, 2018; Gaston et al., 2017). ALAN is currently widespread worldwide, with 83% of the world's population living under a sky affected by ALAN (Davies & Smyth, 2018; Falchi et al., 2016). Between 2011 and 2022, the sky's brightness increased on average by 9.6 % per year (Kyba et al., 2023). Among the surfaces exposed to ALAN, coastal areas are strongly impacted, and marine protected areas are not spared. Concerning the native European oyster *O. edulis*' habitats, i.e. Europe's coastline areas, 54 % are estimated to be light-polluted (Davies et al., 2014, 2016). It is now a consensus that ALAN poses a severe threat to biodiversity

and ecosystems, mainly because of the wrong photic signal it provides to biological clockworks (Davies et al., 2017; Gaston et al., 2017; Hölker et al., 2021; Sanders et al., 2021). In a context where daily light cycles are no longer reliable due to ALAN, processing a labile daily rhythm, as observed in *O. edulis*, which may be easily disrupted by photic cues, may disadvantage the organism in maintaining synchrony with its environment.
Consequently, as *O. edulis* is a sessile and benthic organism that cannot escape ALAN, a decrease in its fitness is highly anticipated in coastal shallow water environments. In this study, when oysters were exposed to a realistic and low level of ALAN (5 lx), no abolishment of the daily rhythm was observed, as seen under dim L:L. However, the results indicated a disappearance of the daily rhythm for VOD, with a shift of the maximum VOD from nocturnal to diurnal. These findings align with the hypothesis of the high susceptibility of *O. edulis* to ALAN, due to a clock characterized as labile. Such sensitivity to ALAN has recently been highlighted in its close congener, the oyster *C. gigas*, for which the circadian clock was also described as labile (Mat et al., 2012). In this species, the behavioral daily rhythm, along with the expression of circadian clock and clock-associated genes, can be impaired by ALAN, even with a low exposure of 0.1 lx (Botté, Payton, & Tran, 2023a). Moreover, it has been shown that the eyeless oyster *C. gigas* responds differently to ALAN depending on the light quality (Botté, Payton, Lefeuvre, et al., 2023) and exposure modality (Botté, Payton, & Tran, 2023b).

This study contributes to filling a knowledge gap in understanding of the biology of *O. edulis* by characterizing the plasticity of its circadian clock. This adaptive (i.e. labile and plastic) clockwork may explain the wide historical distribution of *O. edulis* throughout the European waters from the Mediterranean Sea to Norway and from shallow coastal waters to depth of 80 m in sublittoral and offshore environments (Pogoda et al., 2019; Pouvreau et al., 2023; Thurstan et al., 2023). Today, over 85 % of native oyster reef habitat is lost across Europe. The Native Oyster Restoration Alliance (NORA, https://noraeurope.eu/) supports knowledge exchange for the restoration and the protection of this endangered species, a key organism for biogenic reef habitats in European coastal ecosystems (Pogoda, 2019; Pouvreau et al., 2023; Thurstan et al., 2023). Light pollution should be considered as an increasing anthropogenic pressure, and a relevant parameter potentially affecting nearshore native oyster restoration measures. To our knowledge, this is the first study to assess the endogenous clock and behavioral rhythms in the native European oyster. Nevertheless, further analyses are needed to better characterize the circadian rhythm in terms of temperature compensation, which is a fundamental property of circadian rhythms. This property would inform us about

this species adaptability in the context of climate change. Additionally, further chronobiological experiences are required to demonstrate that a functional circadian clock of *O. edulis* is entrainable by daily light dark cycles and responsible of apparent daily rhythms. The behavioral rhythms of this species remain to be studied in its natural environment to compare with laboratory results and to determine how *O. edulis* copes with the effects of climate change and anthropogenic pressures such as ALAN. Understanding of how environmental changes influence the behavioral rhythms of *O. edulis* (e.g., the combination of both natural and anthropogenic drivers) is a key point for succeeding in its recovery.

Acknowledgments

A. Le Moal was supported by a grant awarded to B. Meyer and B. Pogoda via AWI Inspires - International Science Program for Integrative Research in Earth Systems. The study contributes to the Helmholtz Research Program "Changing Earth-Sustaining our future" of the research field Earth and Environment of the Helmholtz Association, Topic 6, Subtopic 6.1. and 6.2 as well as Topic 4, Suptopic 4.2. The project was supported by the "PHC PROCOPE" program via the project "OysterClock" (project-ID: 57701739), funded by the French Ministry for Europe and Foreign Affairs, the French Ministry for Higher Education and Research and the German Academic Exchange Service (DAAD). The was also supported by the French National Research Agency (ANR), LUCIOLE project (ANR-22-CE34- 0010-01).
We thank Audrey Botté and Yannick Geerebaert for their help during the setup of the experiment. We thank Kristin Tessmar-Raible and Stephane Pouvreau for their valuable discussions.

Data availability

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

References

Abhilash, L., Shindey, R., & Sharma, V. K. (2017). To be or not to be rhythmic? A review of studies on organisms inhabiting constant environments. *Biological Rhythm Research*, *48*(5), 677–691. https://doi.org/10.1080/09291016.2017.1345426

Ameyaw-Akumfi, C., & Naylor, E. (1987). Temporal patterns of shell-gape in Mytilus edulis. *Marine Biology*, *95*(2), 237–242. https://doi.org/10.1007/BF00409011

Andreatta, G., & Tessmar-Raible, K. (2020). The Still Dark Side of the Moon: Molecular Mechanisms of Lunar-Controlled Rhythms and Clocks. *Journal of Molecular Biology*, *432*(12), 3525–3546. https://doi.org/10.1016/j.jmb.2020.03.009

Aschoff, J. (1981). Freerunning and Entrained Circadian Rhythms. In J. Aschoff (Ed.), *Biological Rhythms* (pp. 81–93). Springer US. https://doi.org/10.1007/978-1-4615-6552-9_6

Beaugrand, G., Conversi, A., Atkinson, A., Cloern, J., Chiba, S., Fonda-Umani, S., Kirby, R. R., Greene, C. H., Goberville, E., Otto, S. A., Reid, P. C., Stemmann, L., & Edwards, M. (2019). Prediction of unprecedented biological shifts in the global ocean. *Nature Climate Change*, *9*(3), 237–243. https://doi.org/10.1038/s41558-019-0420-1

Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators: Lessons from diverse organisms. *Nature Reviews Genetics*, *6*(7), 544–556. https://doi.org/10.1038/nrg1633

Bertolini, E., Schubert, F. K., Zanini, D., Sehadová, H., Helfrich-Förster, C., & Menegazzi, P. (2019). Life at High Latitudes Does Not Require Circadian Behavioral Rhythmicity under Constant Darkness. *Current Biology*, *29*(22), 3928-3936.e3. https://doi.org/10.1016/j.cub.2019.09.032

Bingham, C., Arbogast, B., Guillaume, G. C., Lee, J. K., & Halberg, F. (1982). Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*, *9*(4), 397–439.

Bloch, G., Barnes, B. M., Gerkema, M. P., & Helm, B. (2013). Animal activity around the clock with no overt circadian rhythms: Patterns, mechanisms and adaptive value. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20130019. https://doi.org/10.1098/rspb.2013.0019

Botté, A., Payton, L., Lefeuvre, E., & Tran, D. (2023). Is part-night lighting a suitable mitigation strategy to limit Artificial Light at Night effects on the biological rhythm at the behavioral and molecular scales of the oyster *Crassostrea gigas*? *Science of The Total Environment*, *905*, 167052. https://doi.org/10.1016/j.scitotenv.2023.167052

Botté, A., Payton, L., & Tran, D. (2023a). Artificial light at night at environmental intensities disrupts daily rhythm of the oyster *Crassostrea gigas*. *Marine Pollution Bulletin*, *191*, 114850. https://doi.org/10.1016/j.marpolbul.2023.114850

Botté, A., Payton, L., & Tran, D. (2023b). The effects of artificial light at night on behavioral rhythm and related gene expression are wavelength dependent in the oyster Crassostrea gigas. *Environmental Science and Pollution Research*, *30*(57), 120375–120386. https://doi.org/10.1007/s11356-023-30793-1

Box, G. E. P., Jenkins, G. M., & Reinsel, G. C. (1994). *Time series analysis: Forecasting and control* (Third edition). Prentice Hall. http://www.gbv.de/dms/bowker/toc/9780130607744.pdf

Bradshaw, W. E., & Holzapfel, C. M. (2010). Light, Time, and the Physiology of Biotic Response to Rapid Climate Change in Animals. *Annual Review of Physiology*, *72*(Volume 72, 2010), 147–166. https://doi.org/10.1146/annurev-physiol-021909-135837

Davies, T. W., Bennie, J., Cruse, D., Blumgart, D., Inger, R., & Gaston, K. J. (2017). Multiple night-time light-emitting diode lighting strategies impact grassland invertebrate assemblages. *Global Change Biology*, *23*(7), 2641–2648. https://doi.org/10.1111/gcb.13615

Davies, T. W., Duffy, J. P., Bennie, J., & Gaston, K. J. (2014). The nature, extent, and ecological implications of marine light pollution. *Frontiers in Ecology and the Environment*, *12*(6), 347–355. https://doi.org/10.1890/130281

Davies, T. W., Duffy, J. P., Bennie, J., & Gaston, K. J. (2016). Stemming the Tide of Light Pollution Encroaching into Marine Protected Areas. *Conservation Letters*, *9*(3), 164–171. https://doi.org/10.1111/conl.12191

Davies, T. W., & Smyth, T. (2018). Why artificial light at night should be a focus for global change research in the 21st century. *Global Change Biology*, *24*(3), 872–882. https://doi.org/10.1111/gcb.13927

Dodd, A. N., Salathia, N., Hall, A., Kévei, E., Tóth, R., Nagy, F., Hibberd, J. M., Millar, A. J., & Webb, A. A. R. (2005). Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and Competitive Advantage. *Science*, *309*(5734), 630–633. https://doi.org/10.1126/science.1115581

Emerson, K. J., Bradshaw, W. E., & Holzapfel, C. M. (2008). Concordance of the circadian clock with the environment is necessary to maximize fitness in natural populations. *Evolution; International Journal of Organic Evolution*, *62*(4), 979–983. https://doi.org/10.1111/j.1558-5646.2008.00324.x

Enright, J. T. (1976). Plasticity in an isopod's clockworks: Shaking shapes form and affects phase and frequency. *Journal of Comparative Physiology*, *107*(1), 13–37. https://doi.org/10.1007/BF00663916

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C. M., Elvidge, C. D., Baugh, K., Portnov, B. A., Rybnikova, N. A., & Furgoni, R. (2016). The new world atlas of artificial night sky brightness. *Science Advances*, *2*(6), e1600377. https://doi.org/10.1126/sciadv.1600377

García-March, J. R., Sanchís Solsona, M. Á., & García-Carrascosa, A. M. (2008). Shell gaping behaviour of Pinna nobilis L., 1758: Circadian and circalunar rhythms revealed by in situ monitoring. *Marine Biology*, *153*(4), 689–698. https://doi.org/10.1007/s00227-007-0842-6

Gaston, K. J., Davies, T. W., Nedelec, S. L., & Holt, L. A. (2017). Impacts of Artificial Light at Night on Biological Timings. *Annual Review of Ecology, Evolution, and Systematics*, *48*(Volume 48, 2017), 49–68. https://doi.org/10.1146/annurev-ecolsys-110316-022745

Gwinner, E., & Brandstatter, R. (2001). Complex bird clocks. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *356*(1415), 1801–1810. https://doi.org/10.1098/rstb.2001.0959

Haelters, J., & Kerckhof, F. (2009). *Background document for Ostrea edulis and Ostrea edulis bed*. OSPAR Commission – Biodiversity Series, pp. 1–22. Anonymous, London.

Häfker, N. S., Andreatta, G., Manzotti, A., Falciatore, A., Raible, F., & Tessmar-Raible, K. (2023). Rhythms and Clocks in Marine Organisms. *Annual Review of Marine Science*, *15*(Volume 15, 2023), 509–538. https://doi.org/10.1146/annurev-marine-030422-113038

Helm, B., Ben-Shlomo, R., Sheriff, M. J., Hut, R. A., Foster, R., Barnes, B. M., & Dominoni, D. (2013). Annual rhythms that underlie phenology: Biological time-keeping meets environmental change. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20130016. https://doi.org/10.1098/rspb.2013.0016

Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., & Bloch, G. (2017). Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1734), 20160246. https://doi.org/10.1098/rstb.2016.0246

Hölker, F., Bolliger, J., Davies, T. W., Giavi, S., Jechow, A., Kalinkat, G., Longcore, T., Spoelstra, K., Tidau, S., Visser, M. E., & Knop, E. (2021). 11 Pressing Research Questions on How Light Pollution Affects Biodiversity. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.767177

Kharouba, H. M., & Wolkovich, E. M. (2020). Disconnects between ecological theory and data in phenological mismatch research. *Nature Climate Change*, *10*(5), Article 5. https://doi.org/10.1038/s41558-020-0752-x

Klotter, K. (1960). General properties of oscillating systems. *Cold Spring Harbor Symposia on Quantitative Biology*, *25*, 185–187. https://doi.org/10.1101/sqb.1960.025.01.016

Krittika, S., & Yadav, P. (2020). Circadian clocks: An overview on its adaptive significance. *Biological Rhythm Research*, *51*(7), 1109–1132. https://doi.org/10.1080/09291016.2019.1581480

Kyba, C. C. M., Altıntaş, Y. Ö., Walker, C. E., & Newhouse, M. (2023). Citizen scientists report global rapid reductions in the visibility of stars from 2011 to 2022. *Science (New York, N.Y.)*, *379*(6629), 265–268. https://doi.org/10.1126/science.abq7781

Last, K. S., Bailhache, T., Kramer, C., Kyriacou, C. P., Rosato, E., & Olive, P. J. W. (2009). Tidal, daily, and lunar-day activity cycles in the marine polychaete Nereis virens. *Chronobiology International*, *26*(2), 167–183. https://doi.org/10.1080/07420520902774524

Le Moal, A., Payton, L., Andrade, H., Camus, L., Ballantine, C., Ciret, P., & Tran, D. (2023). Blue mussels' valve behavior exhibits daily and lunar rhythms during the high Arctic polar day. *Marine Biology*, *170*(9), 113. https://doi.org/10.1007/s00227-023-04257-6

Mat, A., Massabuau, J.-C., Ciret, P., & Tran, D. (2012). Evidence for a Plastic Dual Circadian Rhythm in the Oyster Crassostrea gigas. *Chronobiology International*, *29*(7), 857–867. https://doi.org/10.3109/07420528.2012.699126

Mat, A., Massabuau, J.-C., Ciret, P., & Tran, D. (2014). Looking for the clock mechanism responsible for circatidal behavior in the oyster Crassostrea gigas. *Marine Biology*, *161*(1), 89–99. https://doi.org/10.1007/s00227-013-2317-2

Mermet, J., Yeung, J., & Naef, F. (2017). Systems Chronobiology: Global Analysis of Gene Regulation in a 24-Hour Periodic World. *Cold Spring Harbor Perspectives in Biology*, *9*(3), a028720. https://doi.org/10.1101/cshperspect.a028720

Naylor, E. (2010). *Chronobiology of Marine Organisms*. Cambridge University Press. https://doi.org/10.1017/CBO9780511803567

Palmer, J. D. (1995). Review of the Dual-Clock Control of Tidal Rhythms and the Hypothesis that the Same Clock Governs Both Circatidal and Circadian Rhythms. *Chronobiology International*, *12*(5), 299–310. https://doi.org/10.3109/07420529509057279

Palmer, J. D. (2000). The clocks controlling the tide-associated rhythms of intertidal animals. *BioEssays*, *22*(1), 32–37. https://doi.org/10.1002/(SICI)1521-1878(200001)22:1<32::AID-BIES7>3.0.CO;2-U

Payton, L., & Tran, D. (2019). Moonlight cycles synchronize oyster behaviour. *Biology Letters*, *15*(1), 20180299. https://doi.org/10.1098/rsbl.2018.0299

Per Huffeldt, N. (2020). Photic Barriers to Poleward Range-shifts. *Trends in Ecology & Evolution*, *35*(8), 652–655. https://doi.org/10.1016/j.tree.2020.04.011

Pogoda, B. (2019). Current Status of European Oyster Decline and Restoration in Germany. *Humanities*, *8*(1), Article 1. https://doi.org/10.3390/h8010009

Pogoda, B., Boudry, P., Bromley, C., Cameron, T. C., Colsoul, B., Donnan, D., Hancock, B., Hugh-Jones, T., Preston, J., Sanderson, W. G., Sas, H., Brown, J., Bonacic, K., von Nordheim, H., & zu Ermgassen, P. S. E. (2020). NORA moving forward: Developing an oyster restoration network in Europe to support the Berlin Oyster Recommendation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2031–2037. https://doi.org/10.1002/aqc.3447

Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., Sanderson, W., & Nordheim, H. von. (2019). The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation: Bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquatic Living Resources*, *32*, 13. https://doi.org/10.1051/alr/2019012

Pouvreau, S., Lapègue, S., Arzul, I., & Boudry, P. (2023). Fifty years of research to counter the decline of the European flat oyster (Ostrea edulis): A review of French achievements and prospects for the restoration of remaining beds and revival of aquaculture production. *Aquatic Living Resources*, *36*, 13. https://doi.org/10.1051/alr/2023006

Sanders, D., Frago, E., Kehoe, R., Patterson, C., & Gaston, K. J. (2021). A meta-analysis of biological impacts of artificial light at night. *Nature Ecology & Evolution*, *5*(1), 74–81. https://doi.org/10.1038/s41559-020-01322-x

Scargle, J. D. (1982). Studies in astronomical time series analysis. II. Statistical aspects of spectral analysis of unevenly spaced data. *The Astrophysical Journal*, *263*, 835–853. https://doi.org/10.1086/160554

Smyth, T. J., Wright, A. E., McKee, D., Tidau, S., Tamir, R., Dubinsky, Z., Iluz, D., & Davies, T. W. (2021). A global atlas of artificial light at night under the sea. *Elementa: Science of the Anthropocene*, *9*(1), 00049. https://doi.org/10.1525/elementa.2021.00049

Tessmar-Raible, K., Raible, F., & Arboleda, E. (2011). Another place, another timer: Marine species and the rhythms of life. *BioEssays*, *33*(3), 165–172. https://doi.org/10.1002/bies.201000096

Thurstan, R. H., McCormick, H., Preston, J., Ashton, E. C., Bennema, F. P., Cetinić, A. B., Brown, J. H., Cameron, T. C., Costa, F. da, Donnan, D., Ewers, C., Fortibuoni, T., Galimany, E., Giovanardi, O., Grancher, R., Grech, D., Hayden-Hughes, M., Helmer, L., Jensen, K. T., zu Ermgassen, P. S. (2023). *Historical dataset details the distribution, extent and form of lost Ostrea edulis reef ecosystems.* https://ecoevorxiv.org/repository/view/6359/

Tonk, L., Witbaard, R., Dalen, P. van, Cheng, C. H., & Kamermans, P. (2023). Applicability of the gape monitor to study flat oyster (Ostrea edulis) feeding behaviour. *Aquatic Living Resources*, *36*(6), 6. https://doi.org/10.1051/alr/2022021

Tran, D., Andrade, H., Camus, L., Leopold, P., Ballantine, C., Berge, J., Durier, G., Sow, M., & Ciret, P. (2023). Evidence of separate influence of moon and sun on light synchronization of mussel's daily rhythm during the polar night. *iScience*, *26*(3). https://doi.org/10.1016/j.isci.2023.106168

Tran, D., Nadau, A., Durrieu, G., Ciret, P., Parisot, J.-P., & Massabuau, J.-C. (2011). Field Chronobiology of a Molluscan Bivalve: How the Moon and Sun Cycles Interact to Drive Oyster Activity Rhythms. *Chronobiology International*, *28*(4), 307–317. https://doi.org/10.3109/07420528.2011.565897

Tran, D., Perrigault, M., Ciret, P., & Payton, L. (2020). Bivalve mollusc circadian clock genes can run at tidal frequency. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192440. https://doi.org/10.1098/rspb.2019.2440

Tran, D., Sow, M., Camus, L., Ciret, P., Berge, J., & Massabuau, J.-C. (2016). In the darkness of the polar night, scallops keep on a steady rhythm. *Scientific Reports*, *6*(1), 32435. https://doi.org/10.1038/srep32435

Webb, H. M. (1976). Interactions of daily and tidal rhythms. Biological rhythms in the marine environment. *University of South Carolina Press, Columbia*, 129–135.

Yerushalmi, S., & Green, R. M. (2009). Evidence for the adaptive significance of circadian rhythms. *Ecology Letters*, *12*(9), 970–981. https://doi.org/10.1111/j.1461-0248.2009.01343.x

4. Article II - Identification and expression of circadian clock genes

Identification and expression of circadian clock genes in the European flat oyster Ostrea edulis

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Published in Royal Society Open Science - https://doi.org/10.1098/rsos.250970

Abstract

Circadian clocks enable organisms to synchronize their biological processes to environmental daily cycles, thereby increasing species fitness. Components of the molecular clock are conserved across many taxa, though their structure and function can vary. In this study, we aim to investigate the circadian clock of the European flat oyster *Ostrea edulis*, an endangered species with a pivotal role in biogenic reef ecosystems. Phylogenetic and protein domain analyses were performed to identify orthologs of core clock and clock-associated genes (*OeClock*, *OeBmal1*, *OePeriod*, *OeTimeless*, *OeCryptochrome2, OeRev-erb*, *OeRor*, *OeDoubletime*, *OeClockWorkOrange*, *OeShaggy*), a gene related to melatonin synthesis (*OeHiomt*), and genes involved in light perception (*OeCryptochrome1*, *OeOpsin4*). As a functional output of the clock, we observed daily and circadian rhythms in valve behavior recorded under light:dark and constant dark conditions. In parallel, gene expression analyses in two tissues under similar light regimes revealed tissue-specific rhythms, suggesting the presence of a functional and plastic endogenous circadian system in *O. edulis*. These findings offer a first molecular work for deeper exploration of the circadian clock functioning in *O. edulis*, providing essential resources to investigate its evolutionary adaptation to cyclic environments, and inform restoration and conservation strategies of this threatened species.

Keywords: Circadian clock, *Ostrea edulis,* biological rhythm, bivalve, phylogenetic tree, gene expression, valve behavior

Introduction

Life on Earth has been confronted with environmental cycles of varying periods, influenced by the interactions between the sun, the Earth, and moon trajectories (Andreatta & Tessmar-Raible, 2020). In order to adapt and survive in these dynamic environments, organisms have developed an endogenous timing mechanism. This internal clock enables organisms to synchronize with their environment and anticipate daily environmental fluctuations, thereby regulating various metabolic, physiological, and behavioral processes (Bell-Pedersen et al., 2005). Research across various taxa, including vertebrates (E. E. Zhang & Kay, 2010), insects (Dubruille & Emery, 2008; Hardin, 2005), and crustaceans (L. Zhang et al., 2013) reveals that while the molecular architecture of circadian clocks may differ, fundamental clock genes are mostly conserved, although their functions and interactions may vary across phyla (Yuan et al., 2007). The molecular mechanisms of a circadian clock involves negative and positive transcriptional and translational feedback loop interactions among clock genes and their proteins (Cermakian & Sassone-Corsi, 2000; Dunlap, 1999; Partch et al., 2014). This molecular system maintains a circadian rhythm (literally *about a day*) even without external stimuli, i. e., under free-running conditions (Box et al., 1994). To stay in sync with external cycles, the clock is entrained by environmental cues called *zeitgebers* (time givers) (Dubruille & Emery, 2008). The daily light/dark cycle is the primary *zeitgeber* for the circadian clock, as it is the most reliable environmental cue for daily cycles (Goto, 2013; Herrero & Lepesant, 2014). Marine organisms exist in a complex environment influenced not only by the daily light/dark cycle but also by cycles of shorter or longer durations, such as tidal, lunar/semi-lunar or seasonal cycles (Naylor, 2010; Palmer, 1995). The functional mechanisms of molecular clocks in marine invertebrates remain poorly charachterized. In bivalves, studies on species such as the Pacific oyster Crassostrea gigas (Perrigault & Tran, 2017; Tran et al., 2020), the blue mussel Mytilus edulis (Chapman et al., 2017), and the Arctic scallops Chlamys islandica (Perrigault et al., 2020) show the presence of orthologues of core and related circadian clock genes, indicating a high degree of conservation.

The European native oyster, *O. edulis* was formerly found throughout Europe, from intertidal coastal areas to deeper offshore waters (Pogoda, 2019; Pouvreau et al., 2023; Zu Ermgassen et al., 2020). Today, this ecological key species is under severe threat due to overfishing, habitat degradation, habitat loss, pollution and invasive diseases (e.g. *Marteilia*, *Bonamia*) (Pogoda et al., 2020). Native oysters are highly gregarious, meaning that oyster larvae prefer to settle where other oysters are present, allowing them to form

a structured biogenic reef habitat for different species (Colsoul et al., 2021). Native oyster habitats, known as oyster reefs, provide key ecosystem functions and enhance biodiversity (Haelters & Kerckhof, 2009). Therefore, the conservation of European flat oyster populations has recently become a focus of ecological restoration efforts (Pogoda et al., 2019). Despite the ecological importance of O. edulis, little is known about the environmental drivers of its biological rhythms, which limits the understanding of its resilience to climate-induced environmental changes and, consequently, its conservation efforts. A recent study highlighted that this oyster exhibits a daily rhythm of valve behavior synchronized by light/dark cycles, that persists under constant darkness (Le Moal et al., 2025). These findings demonstrate the presence of a functional endogenous clock mechanism that is highly synchronizable by light. However, to date, the molecular clock components and circadian clockwork of *O. edulis* have yet to be investigated. While mollusc and bivalve genome sequencing and assembly have traditionally been hindered by technical and genetic complexities, as these organisms often possess large, highly repetitive, and heterozygous genomes (Adema, 2021; Davison & Neiman, 2021), a recent study has integrated multiple technologies to produce a high-quality chromosome-level reference genome for the European flat oyster, paving the way to explore its molecular circadian clockwork (Gundappa et al., 2022).

The present study focuses on identifying the circadian clock genes responsible for generating and synchronizing rhythms in O. edulis. The objective of the study is to identify 13 orthologous genes that are putatively involved in the clock machinery, including 10 circadian clock genes (*OeClock*, *OeBmal1*, *OePer1*, *OeTim1*, *OeCry2*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeCwo*, *OeShag*), 1 gene involved in melatonin synthesis *(OeHiomt*), and 2 photoreceptor genes (*OeCryptochrome1 and OeOpn4).* Furthermore, the gene expression patterns in gills and labial palps are investigated under light/dark cycles and constant darkness. Concurrently, we monitor and analyze the daily and circadian rhythms of valve behavior, which are an output of the circadian clockwork. Based on these analyses, we hypothesize that *O. edulis* possesses a functional endogenous circadian system, exhibiting tissue-specific gene expression patterns and synchronizing behavioral rhythms. Therefore, the goal of this study is first to characterize the molecular components of the circadian clock in *O. edulis*, and second to explore their potential involvement in generating behavioral rhythms, providing preliminary insight into the temporal organization of biological rhythms in this ecologically important and threatened species.

Material and methods

Clock genes mining

A bioinformatic analysis was conducted to identify genes of interest involved or related to the circadian clock machinery. Gene mining was performed using the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) along with the reference genome assembly xbOstEdul1.1 (NCBI RefSeq assembly GCF_947568905.1, (Gundappa et al., 2022)). For genes that were already predicted and annotated (*OeClock*, *OeBmal1*, *OeTimless (OeTim1)*, *OeRor*, *OeDoubletime (OeDbt)*, *OeShaggy (OeShag)*, *OeCryptochrome1 (OeCry1)* and *OeOpsin4 (OeOpn4)*), a protein-protein BLAST (blastp) was performed based on corresponding proteins described in other species, and the corresponding protein sequences were downloaded in FASTA format for further analysis and validation. For non-annotated genes (*OePeriod (OePer1)*, *OeCryptochrome2 (OeCry2)*, *OeRev-erb*, *OeClockWorkOrange (OeCwo), and OeHiomt*), a protein-protein BLAST (blastp) was conducted based on corresponding proteins described in other species. Blastp searches were filtered with an E-value < 1e-5. Sequences with >30% identity were retained, or >25% in rare cases when conserved domains were confirmed. Partial, likely non-functional, or misannotated sequences were excluded based on sequence integrity and domain structure. To ensure that the identified sequences correspond to the genes of interest, once all genes were identified, the sequences were analyzed using InterProScan (https://www.ebi.ac.uk/interpro/) to investigate specific protein domains and validate their relevance to the clock mechanism under investigation. Accession numbers for identified genes are available in Supplementary table S9.2.

Phylogenetic Tree Construction and representation

DNA sequences were aligned using the MUSCLE method implemented in MEGA X (https://www.megasoftware.net/). To enhance alignment quality, Gblocks (http://phylogeny.lirmm.fr/phylo_cgi/) was employed to trim the alignment, ensuring the removal of poorly aligned positions and divergent regions. Phylogenetic trees were constructed in MEGA X based on a consensus sequence common to all analyzed species, incorporating the protein domains of interest. Model selection for each tree was performed using the Maximum Likelihood (ML) approach within MEGA X, prioritizing models with the highest Akaike Information Criterion (AIC) and log-likelihood (InL) scores. Once the optimal model was identified, trees were generated in MEGA X using

the Maximum Likelihood method based on the Jones-Taylor-Thornton matrix model with 1000 bootstrap replicates to assess statistical support and were represented using FigTree software (http://tree.bio.ed.ac.uk/software/figtree/). Two phylogenetic trees were constructed for each gene, the first one includes multiple phyla of the animal kingdom and the second one is at the bivalve level. Phylogenetic trees were constructed only when enough sequences from other species were available. Phylogenetic trees were constructed only when sufficient sequences from other species were available. To ensure broad representation across families, we selected the maximum number of clearly annotated or identified bivalve sequences available in public databases. For Animalia-level trees, representative bivalves were included alongside sequences from other major metazoan lineages, but a limited number of bivalve sequences was retained to avoid overrepresentation and maintain balanced comparisons across phyla. Accession numbers of genes used to construct phylogenetic trees are provides in the electronic supplementary material, table S9.2 for *Ostrea edulis* sequences and dataset S1 (available with the original publication) for other species.

Experimental model

All research conducted in this study adhered to French legislation and followed international ethical standards. The experiments were performed at the Marine Station of Arcachon, France, involving a total of 240 native European oysters (*O. edulis*). The oysters were sourced from a single farm located in Loch Ryan, southwest Scotland (coordinates: 54° 59' 10" N, 5° 03' 18" W), cultivated in subtidal conditions (Rossmore Oysters Ltd.). Before the experiment, the oysters (approximately 8-10 years old, average shell length: 74.8 mm, average shell width: 72.5 mm) were acclimatized for one month in a continuous system utilizing natural seawater from the Arcachon Bay. During this acclimatization period, microalgae (*Chaetoceros calcitrans*) were continuously incorporated into their diet, and the oysters were maintained under natural light conditions with no tidal cycle.

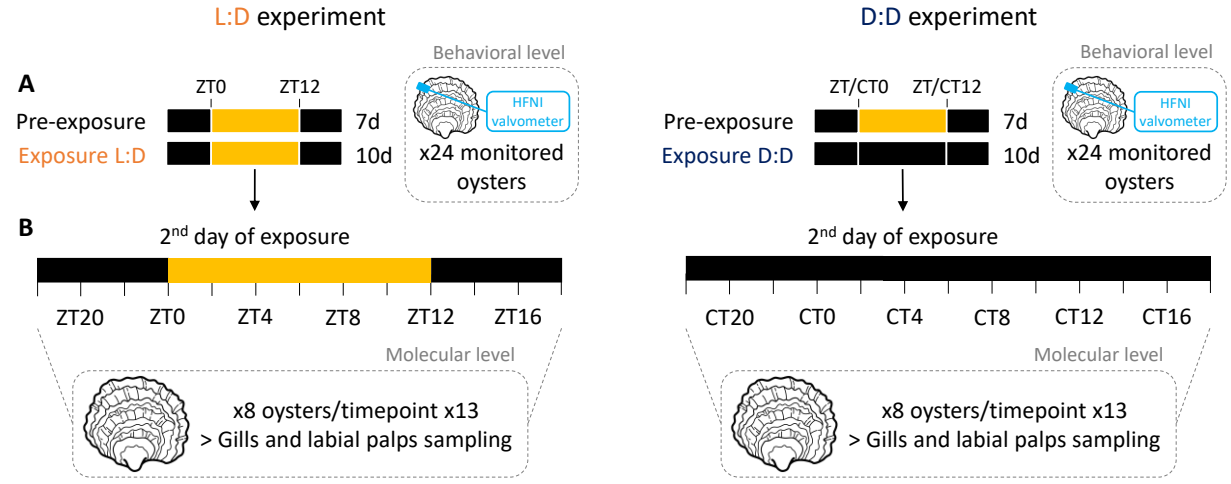


Figure 4.1. Experimental setup. (A) Experimental setup to investigate the rhythm of valve activity behavior of *O. edulis* under L:D 12:12 and D:D. (B) Experimental protocol of time-series sampling for molecular investigation. Black bars = scotophase, yellow bars = photophase. ZT: *zeitgeber* time; CT: circadian time.

Investigations into circadian behaviors and the expression of clock genes were conducted in two 17-day experiments at the Marine Station of Arcachon during winter 2022: the light:dark (L:D) experiment took place from November 12 to November 29, 2022, while the constant darkness (D:D) experiment ran from December 5 to December 21, 2022 (Fig. 4.1). Experiments were performed in winter to minimize confounding effects of reproductive cycles or sex change in *O.edulis* on the measured molecular and behavioral rhythms. In each experiment, 120 oysters were divided into two groups of 60 oysters each, with each group placed in distinct experimental units (EU1 and EU2) measuring 74.8 × 54.8 × 40.8 mm. The seawater in each unit was continuously supplied with oxygenated and filtered seawater (filter diameter < 1 µm) from Arcachon Bay at a flow rate of 350 mL/min/EU, maintaining a constant mean temperature of 15 ± 0.1 °C, a pH of 7.9 ± 0.1, and a salinity of 33.1 ± 0.1 ‰. Prior to the start of the experiment, the oysters were acclimated to the experimental setup for 4 days. Each experiment began with a 7-day pre-exposure phase under a 12-hour light:12-hour dark cycle (L:D), followed by a 10-day L:D exposure phase corresponding to the same 12-hour light:12-hour dark cycle for the L:D experiment and a 10-day constant darkness (D:D) phase for the D:D experiment (Fig. 4.1).

The L:D conditions were achieved using white LED light bars (model MH3SP3 DSunY, 413–685 nm, peak at 553 nm), positioned above each EU. Photophase occurred from

07:00 to 19:00 (all times noted in local time, UTC + 1), with the light intensity varying gradually to mimic the natural light cycle, reaching a maximum light intensity of 1406.1 ± 136.2 lx (mean ± standard error), corresponding to 28.99 µE/m²/s in measured irradiance, between 12:30 and 13:30. Illuminances were measured underwater in each EU at the oysters' depth in lx using a handheld spectroradiometer (Blue-Wave UVN-100, StellarNet Inc.). Equivalent irradiance in µE/m²/s was also measured using a portable radiometer (MICRO Class, Profiling (in-water), E (irradiance) PAR sensor, Biospherical Instrument Inc.). In chronobiological studies, time is expressed as Zeitgeber Time (ZT) during light/dark (LD) conditions, where ZT0 corresponds to the onset of the light phase (lights-on), and as Circadian Time (CT) under constant conditions such as dark/dark (DD), with CT0 aligned to subjective lights-on based on the preceding LD cycle. Thus, in our study, ZT/CT0 corresponds to 07:00, ZT/CT12 to 19:00, and maximal light intensity occurs at ZT6.

Ostrea edulis valve behavior measurement

For each experiment, the valve activity of 24 oysters (12 per EU) was measured using high-frequency non-invasive (HFNI) valvometers. The measurement system consists of a pair of lightweight electrodes (each weighing <100 mg) affixed to each half-shell of the oyster to minimize behavioral disturbance. Each electrode is connected via a flexible cable to the HFNI valvometer, allowing unrestricted movement of the oysters. An electromagnetic current generated between the electrodes enables the measurement of valve activity every 4.8 seconds. Additional details regarding the methodology can be found in Tran et al. (2023) and Le Moal et al. (2023). Raw daily recordings were processed using LabVIEW 8.0 software (National Instruments, Austin, TX, USA). The valve opening amplitude (VOA) was recorded. The individual hourly VOA for each oyster was analysed as a percentage, where 100 % indicates maximum valve opening amplitude throughout the hour and 0 % indicates complete closure. Intermediate values represent varying degrees of opening amplitude. The mean hourly VOA are represented as double-plotted actograms, where each line represents two days (Fig. 4.4). In an actogram, hourly activity levels above the daily median are represented by black bars, while hourly activity levels below the daily median are represented by light grey bars. Hourly individual VOA data are accessible in the electronic supplementary material, dataset S2 (available with the original publication).

Time-series sampling for molecular investigation

The study of molecular rhythms was conducted using time-series sampling of gills and labial palps during both the L:D and D:D experiments. Sampling occurred on the second day of the exposure phase of each experiment, at 2-hour intervals over a 24-hour period across 13 time points (Fig. 4.1). For each time point, 8 oysters were sampled in total (4 in EU1 and 4 in EU2). In the L:D experiment, samples were collected on November 22-23 (10:00-10:00), while in the D:D experiment, sampling took place on December 13-14 (10:00-10:00) (Fig. 4.1). All dissections conducted were performed under red light to minimize disturbance to the oysters. Gills and labial palps tissues were dissected and immediately immersed in RNA Later. Samples were then stored at 4°C for 24 hours before being transferred to -80°C for long-term preservation prior to laboratory analysis.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from gills using Trizol (Invitrogen) and a Direct-zol™ RNA MiniPrep (Zymo Research). The quantity and quality of total RNA were assessed by spectrophotometry (OD230, OD260, OD280). RNA reverse transcription was performed using RevertAid H Minus First Strand cDNA Synthesis Kits (Thermo Scientific).

mRNA expression analysis by Real-Time PCR

Primers for genes of interest (*OeClock*, *OeBmal1*, *OePer1, OeTim1, OeCry2*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeCwo*, *OeShag*, *OeHiomt*, *OeCry1, OeOpn4*) as well as for housekeeping genes (*OeGapdh, OeEf1*, *Oe28S*) were designed using the coding sequences (CDS) of these genes, with the assistance of Primer3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Forward and reverse sequences for the primers are detailed in Supplementary table S9.2. Real-time quantitative PCR (qPCR) was performed with the PowerUp™ SYBR™ Green Master Mix kit (Fisher Scientific). The qPCR reactions were conducted under the following conditions: an initial activation step at 95°C for 2 minutes to activate the Dual-Lock™ DNA polymerase, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute to amplify the target cDNA. At the conclusion of amplification, melting curves were generated by gradually decreasing the temperature from 95°C to 60°C to verify primer specificity. Relative transcript levels of clock and clock-associated genes were determined using the comparative Ct method ($2^{-\Delta Ct}$) as described by Livak and Schmittgen (2001), where ΔCt is calculated as $Ct_{(target\ gene)} - Ct_{(housekeeping\ gene)}$. Gene expression was normalized using the geometric mean of the

housekeeping genes *OeEf1*, *OeGapdh* and *Oe28S*. The choice of these housekeeping genes was supported by previous studies validating their reliability for normalization in gene expression analyses (Du et al., 2013; Morga et al., 2010). Based on stability values (Vandesompele et al., 2002) and MIQE guidelines (Bustin et al., 2009), gene expression was normalized using the geometric mean of the housekeeping genes *OeEf1*, *OeGapdh*, and *Oe28S*. The mean Ct values for these genes during L:D and D:D in gills and labial palps are reported in the supplementary materials (Fig. S9.6). Supplementary dataset S3 (available with the original publication) provides detailed gene expression data in gills and labial palps during L:D and D:D experiments.

Chronobiological analyses

For the behavioral study, chronobiological analyses were conducted on hourly VOA datasets using the software Time Series Analysis Serial Cosinor 6.3. Several steps were necessary to validate a significant rhythm. First, to ensure data quality, the absence of random distribution in the dataset is checked using an autocorrelation diagram. The absence of a stationary phenomenon is assessed through a partial autocorrelation function calculation (Box et al., 1994). Next, the data were examined for periodicity via spectral analyses, specifically the Lomb and Scargle periodogram (Scargle, 1982). This periodogram establishes a significance threshold ($p = 0.95$). Finally, rhythmicity is confirmed by the Cosinor model, utilizing the period identified by the Lomb and Scargle periodogram (Bingham et al., 1982). For a given period, the model is expressed as: $Y(t) = A\cos(\pi t/\tau + \varphi) + M + \varepsilon(t)$, where A represents the amplitude, φ is the acrophase, τ denotes the period, M is the mesor, and ε is the relative error. Two critical tests validate the calculated model and the existence of a rhythm: the elliptic test must be rejected, and the probability for the null amplitude hypothesis must be < 0.05. A chronobiometric parameter was computed: The percent rhythm (PR, %), the percentage of cyclic behavior explained by the model. For each series, the VOA dataset is examined for rhythmicity within the circadian range ($\tau = 24 \pm 4$ h) and in the ultradian range ($\tau < 20$ h). These analyses were conducted at the group level (EU1 + EU2, n = 24 individuals).

Rhythmic analysis of clock genes expression was performed using the R package 'RAIN' (R Core Team, 2023; Thaben & Westermark, 2014). RAIN was specifically designed to detect rhythms in biological datasets independent of waveform utilizing a non-parametric approach (Thaben & Westermark, 2014). Periods are tested in the ultradian (12 h ± 2 h) and daily/circadian (24 h ± 4 h) period ranges and are considered significant

if $p < 0.05$. Significant rhythms in the daily/circadian period ranges are labeled as "daily" under L:D entrainment and "circadian" under D:D.

Results

Identification of genes involved in the circadian machinery of *O. edulis*

Identification of genes and their protein domains in O. edulis

The investigation of the *O. edulis* reference genome assembly (Gundappa et al., 2022), combined with research on protein domains and phylogeny, led to the identification of thirteen orthologs putatively involved or related to the circadian clock machinery. This includes ten circadian clock genes (*OeClock*, *OeBmal1*, *OePer1*, *OeTim1*, *OeCry2*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeCwo*, *OeShag*), one gene involved in melatonin synthesis (*OeHiomt*), and two photoreceptor genes (*OeCry1* and *OeOpn4*) (refer to the complete list with accession numbers in Supplementary table S9.2). The identification and putative function of all thirteen genes of interest were validated based on their protein domain similarities, with the domains of *O. edulis* genes shown in Fig. 4.2 and the corresponding domains from other species presented in Supplementary Figure S9.7.

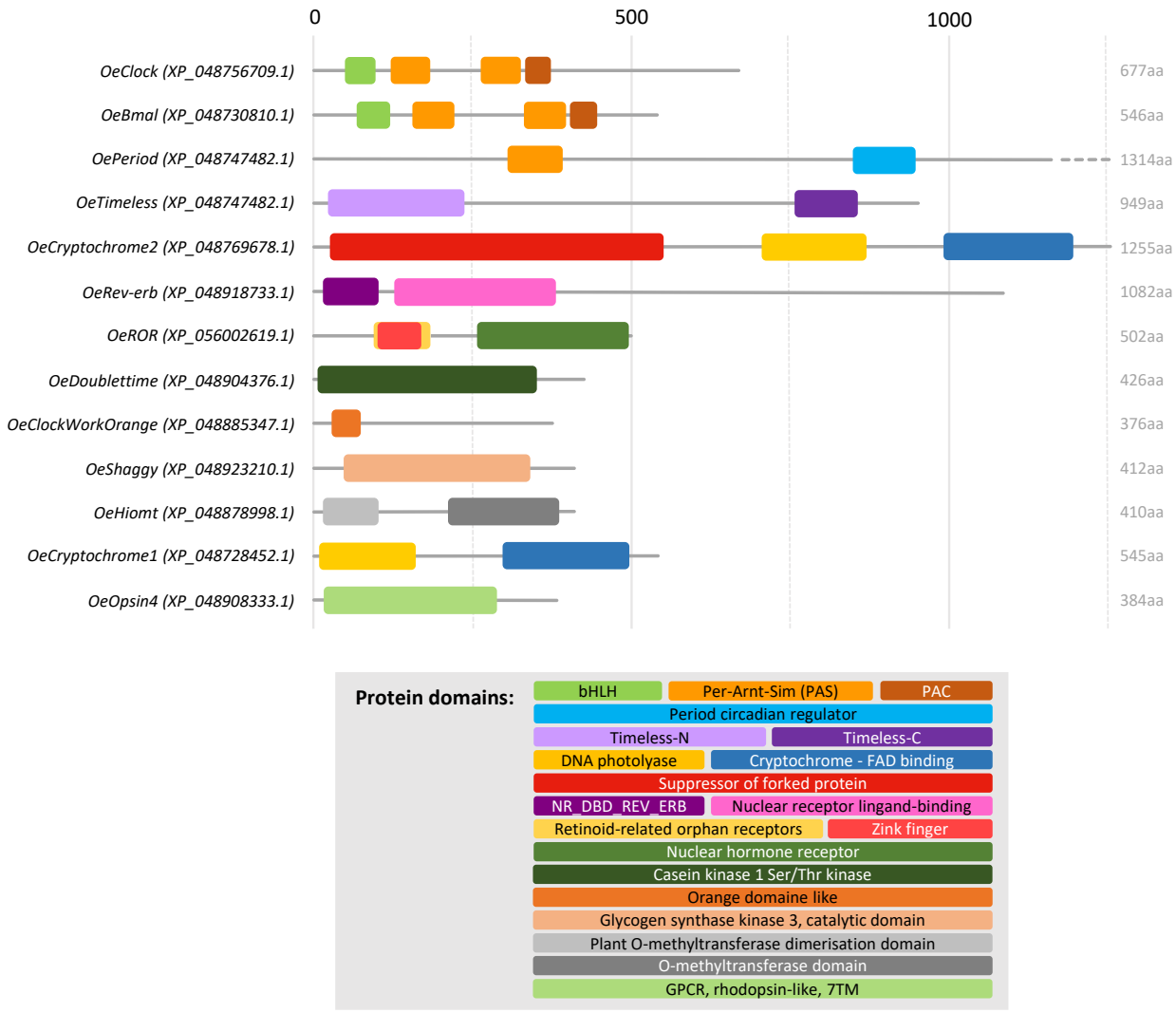


Figure 4.2. Investigation of protein domains in *O. edulis*. Protein domains for *OeClock*, *OeBmal1*, *OePeriod*, *OeTimeless*, *OeCryptochrome2*, *OeRev-erb*, *OeRor*, *OeDoubletime*, *OeClockWorkOrange*, *OeShaggy*, *OeHiomt*, *OeCryptochrome1* and *OeOpsin4*. Grey lines represent the protein length. Colored bars represent protein domains. Protein domains abreviations: basic helix-loop-helix (bHLH) domains, Proline-Rich, Arabinogalactan Proteins, and Conserved Cysteines (PAC) domains, Timeless N-terminal (Timeless-N) domains, Timeless C-terminal (Timeless-C) domains, nuclear receptor DNA-binding (NR_DBD_REV_ERB) domain.

First, we identified OeCLOCK and OeBMAL1 as the putative positive components of the first transcriptional and translational feedback loop in *O. edulis*. A 677 aa *OeClock* sequence (XP_048756709.1) was identified. A 546 aa *OeBmal1* sequence (XP_048730810.1) was also identified. Both sequences contain a basic helix-loop-helix

(bHLH) domain (Fig. 4.2), responsible for interactions with the E-box, and PAS and PAC domains, mediating CLOCK-BMAL1 dimerization (Fig. 4.2). These domains are highly conserved across other animal species and within Bivalvia (Fig. S2), supporting the formation of the OeCLOCK/OeBMAL1 dimer in *O. edulis*.

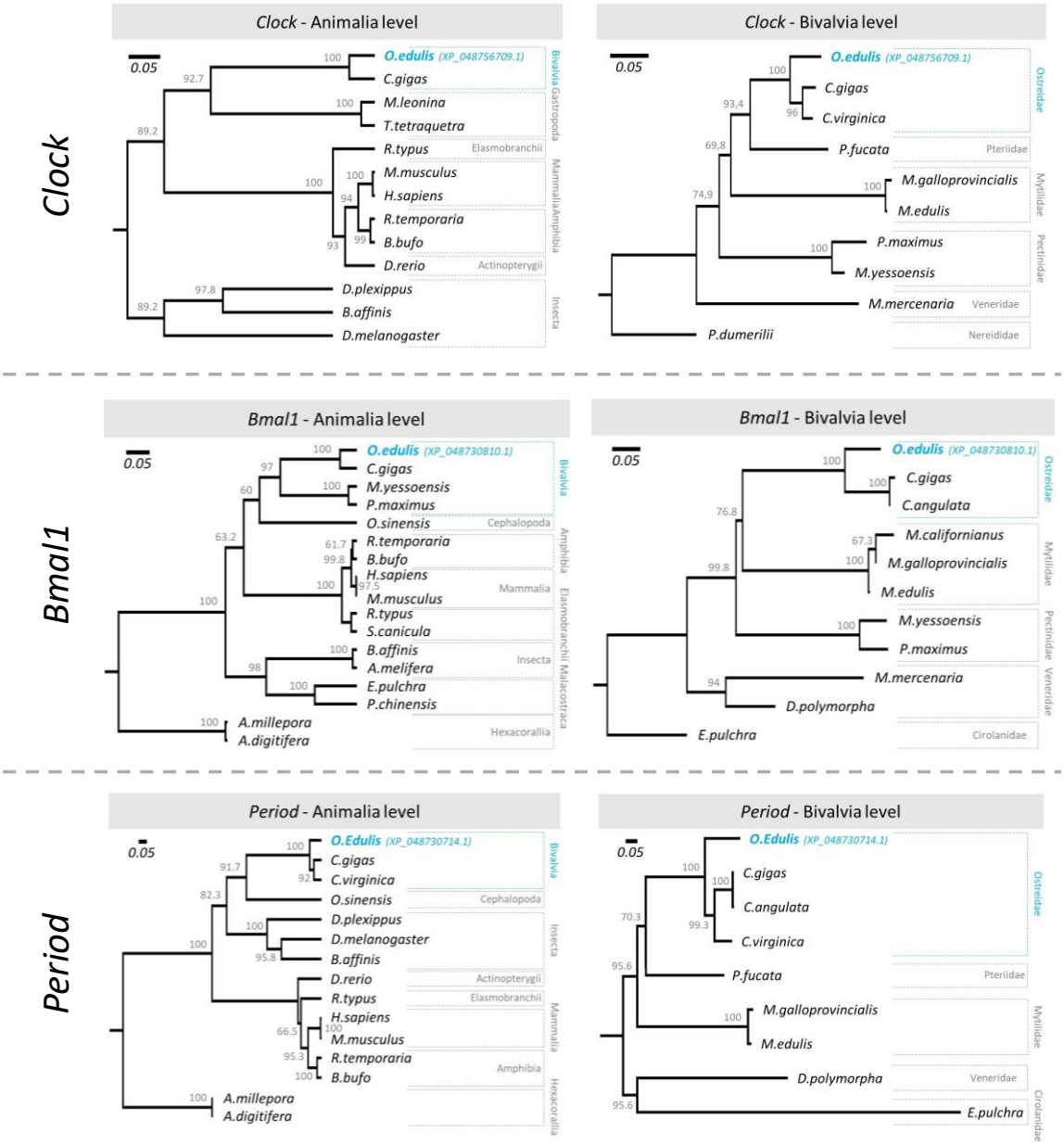
We identified OePER1, OeTIM1, and OeCRY2 as the putative negative elements of the first transcriptional and translational feedback loop in *O. edulis*. The 1314 aa *OePer1* sequence (XP_048730714.1) contained highly conserved protein domain regions with the heterodimerization domains PAS and PAC, and Period protein 2/3 C-terminal region involved in the binding between PER and CRYs in vertebrates (Nangle et al., 2014; Schmalen et al., 2014). In contrast to OeClock and OeBmal1, no bHLH domain was identified in OePer1 (Fig. 4.2), reinforcing its classification as a PERIOD protein that, unlike CLOCK and BMAL, functions in the main transcriptional and translational feedback loop without directly binding to DNA. The 949 aa OeTim1 sequence (XP_048747482.1) and the 1255 aa OeCry2 sequence (XP_048913721.1) contained typical domains (Fig. 4.2), including a FAD-Binding domain, a DNA photolyase domain, and a Suppressor of forked protein (Suf) domain for OeCry2.

We also identified *OeRor* and *OeRev-erb* as orthologs of *ror* (*HR3* in insects) and *rev-erb*, which are known to enhance the robustness of the circadian clock by regulating *Bmal* transcription (Jetten & Joo, 2006; E. E. Zhang & Kay, 2010). The 502 aa *OeRor* sequence was identified (XP_056002619.1), containing Retinoid-related orphan receptors (Fig. 4.2). The 1089 aa sequence of *OeRev-erb* (XP_048918733.1) was identified, containing a nuclear receptor DNA-binding domain (NR_DBD_REV_ERB) and a nuclear receptor ligand-binding domain (Fig. 4.2). Additional clock-related components have been identified: a 426 aa *OeDbt* sequence (XP_048904376.1) contains a Casein kinase 1 Ser/Thr kinase domain, identified as a *doubletime* ortholog known to play a role in the phosphorylation of PERIOD and TIMELESS in *Drosophila* (Nawathean & Rosbash, 2004) . An *OeCwo* sequence (XP_048885347.1), 376 aa in length and containing an Orange domain, is identified as a *cwo* ortholog described as an inhibitor of the CLOCK/BMAL heterodimer, thereby monitoring the amplitude of clock activity (Matsumoto et al., 2007). An *OeShag* sequence (XP_048923210.1), 412 aa in length and containing a Glycogen synthase kinase 3 catalytic domain, was identified (Fig. 4.2).

A 410 aa *OeHiomt* sequence (XP_048878998.1), containing a plant O-methyltransferase dimerization domain and an O-methyltransferase domain, was identified as a *hiomt* ortholog implicated in the synthesis of melatonin (N-acetyl-5-methoxytryptamine) from serotonin (Foulkes et al., 1997).

Finally, photoreceptor genes were identified: the 545 aa OeCry1 sequence (XP_048728452.1) was isolated and contained conserved DNA photolyase and FAD-binding domains involved in the photoreduction of the flavin adenine dinucleotide (FAD) component, characteristic of photolyase/CRY family proteins [46,47] (Fig. 4.2). The *OeOpn4* sequence (XP_048908333.1), 384 aa in length, was identified as an *opsin4* (*melanopsin*) ortholog, a photopigment involved in detecting irradiance levels (Bellingham et al., 2002; Hankins et al., 2008; Peirson & Foster, 2006). It contains a GPCR, rhodopsin-like, 7-transmembrane (7TM) domain characteristic of both *Opn3* and *Opn4* proteins. BLAST analysis further supported its annotation as an *Opn4*-like gene, showing higher similarity to annotated *Opn4* (*melanopsin*) sequences, including Mya arenaria (XP_052777205.1; query cover: 93%, identity: 42%, E-value: 4e-96) and Homo sapiens (AAI13559.1; query cover: 84%, identity: 34%, E-value: 7e-46).

Phylogenetic analyses and similarity to other taxa



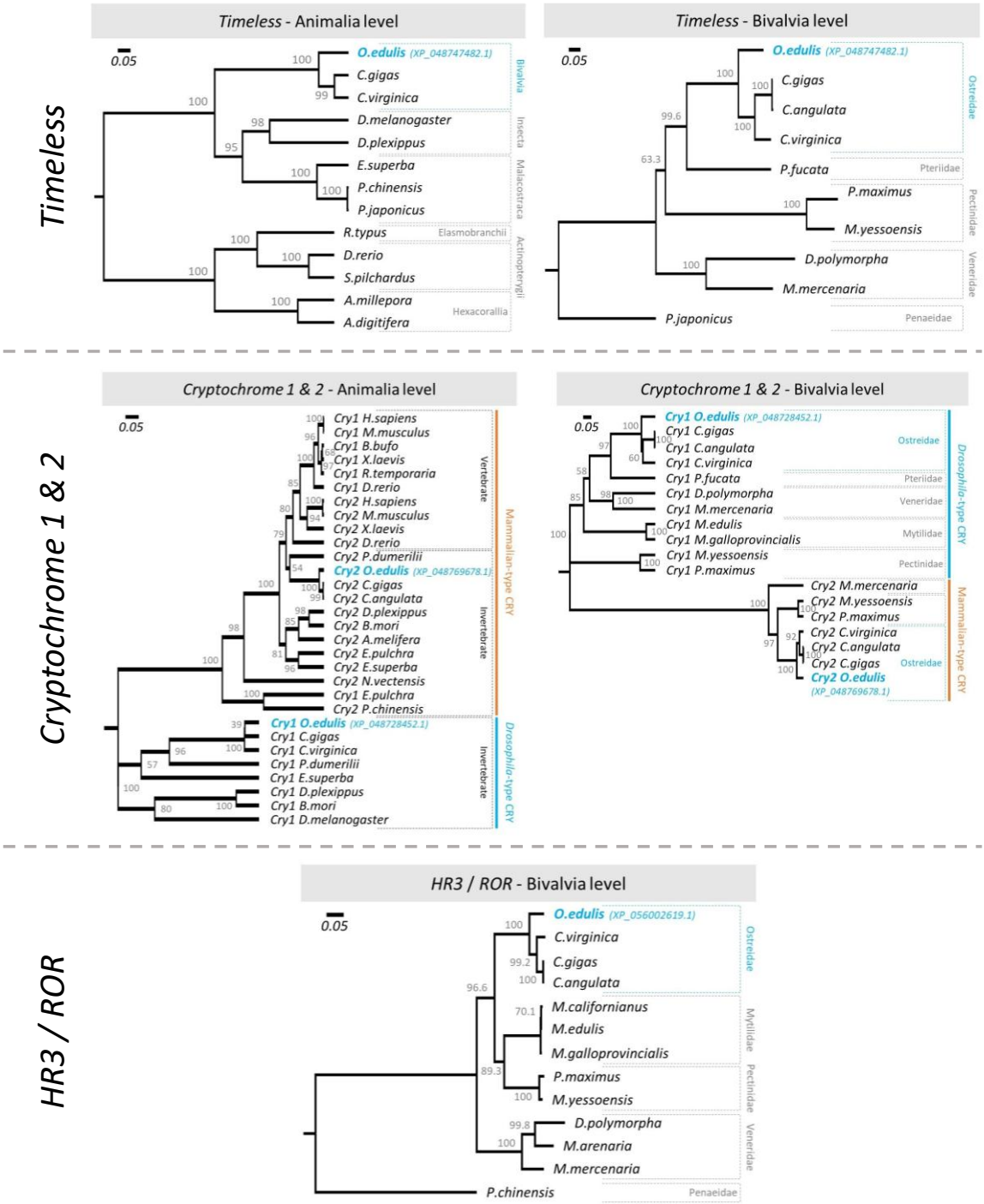


Figure 4.3. Phylogenetic position of *OeClock*, *OeBmal1*, *OePeriod*, *OeTimeless*, *OeCryptochrome1*, *OeCryptochrome2* and *OeRor* within the animal kingdom and the Bivalvia class. Rooted phylogenetic trees of *Clock*, *Bmal1*, *Period*, *Timeless*, *Cryptochrome1*, *Cryptochrome2* and *ROR* sequences within the animal kingdom and the Bivalvia class. The *O.edulis* sequences and the associated family or class are depicted

in clear blue. The numbers on the branches indicate bootstrap values provided by the Maximum Likelihood method with 1000 bootstrap replicates. Abbreviations for each species used in this figure: Acropora digitifera (A. digitifera), Acropora millepora (A. millepora), Apis mellifera (A. mellifera), Bombus affinis (B. affinis), Bombyx mori (B. mori), Bufo bufo (B. bufo), Crassostrea angulata (C. angulata), Crassostrea gigas (C. gigas), Crassostrea virginica (C. virginica), Danaus plexippus (D. plexippus), Danio rerio (D. rerio), Dreissena polymorpha (D. polymorpha), Drosophila melanogaster (D. melanogaster), Euphausia superba (E. superba), Eurydice pulchra (E. pulchra), Homo sapiens (H. sapiens), Mercenaria mercenaria (M. mercenaria), Mizuhopecten yessoensis (M. yessoensis), Mus musculus (M. musculus), Mytilus californianus (M. californianus), Mytilus edulis (M. edulis), Mytilus galloprovincialis (M. galloprovincialis), Mya arenaria (M. arenaria), Nematostella vectensis (N. vectensis), Octopus sinensis (O. sinensis), Ostrea edulis (O. edulis), Pecten maximus (P. maximus), Penaeus chinensis (P. chinensis), Penaeus japonicus (P. japonicus), Pinctada fucata (P. fucata), Platynereis dumerilii (P. dumerilii), Rana temporaria (R. temporaria), Rhincodon typus (R. typus), Sardina pilchardus (S. pilchardus), Tritonia tetraquetra (T. tetraquetra), Xenopus laevis (X. laevis).

Phylogenetic analysis revealed relationships of genes identified in *O. edulis* to orthologs within the animal kingdom and the Bivalvia class. First, for the putative positive components of the first transcriptional and translational feedback loop identified in *O. edulis*, *OeClock* shares closer similarity to mammalian CLOCK than insect sequences (Fig. 4.3); for instance, it shares 55% similarity with the mouse *Mus musculus Clock*-like (NP_001292151.1) and 48% with the fruit fly *D. melanogaster Clock*-like (AAD10630.1). At the Bivalvia level, it shares 82% similarity with the Pacific oyster *C. gigas Clock*-like (XKX15642.1). *OeBmal1* clustered closer to vertebrate-type BMAL1 clades and At the Bivalvia level, it shares 87% similarity with *C. gigas Bmal1*-like (XP_034328823.1) (Fig. 4.3).
Then for the putative negative elements of the first transcriptional and translational feedback loop identified in *O. edulis*, *OePer1* was closer to insect PER orthologs (Fig. 4.3). At the Bivalvia level, it shared 65% and 63% similarity with *Period* orthologs from *C. virginica* (XP_022345652.1) and *C. gigas* (XP_011434453.2), respectively (Fig. 4.3). *OeTim1* was phylogenetically closer to the insect TIM1 orthologs (Fig. 4.3) and shared 69% similarity with a *C. gigas Timeless* ortholog (XP_011434769.2) (Fig. 4.3).
Phylogenetic analysis was also instrumental in distinguishing OeCry1 and OeCry2 and inferring their respective functions. *OeCry2* was found to be closer to Mammalian-type CRY than to *Drosophila*-type CRY (Fig. 4.3), suggesting that *OeCry2 acts* as a

transcriptional repressor in the core clock feedback loop of *O. edulis* (Reppert & Weaver, 2002) (Fig. 4.3). *OeCry1* grouped closer to the *Drosophila*-type CRY1 (Fig. 4.3), suggesting a circadian photoreception role in *O. edulis*. Specifically, we found 86% similarity between *OeCry1* and *C. gigas Cry1*-like (ANJ02841.1) and 56% similarity with the marine annelid *Platynereis dumerilii Cry1*-like (UUF95169.1).
Finally, the phylogenetic tree of *OeRor* at the Bivalvia level revealed important similarity between *O. edulis* and *Ostreidae*, *Mytilidae*, and *Pectinidae*. Specifically, it shared 85% and 65% similarity with the probable *ror* orthologs of *C. gigas* (XP_034321280.1) and *P. maximus* (XP_033724975.1), respectively (Fig. 4.3). The phylogenetic analysis of ROR was restricted to bivalves due to the limited number of confidently identified ROR sequences available at the broader Animalia level.

O. edulis' valve activity

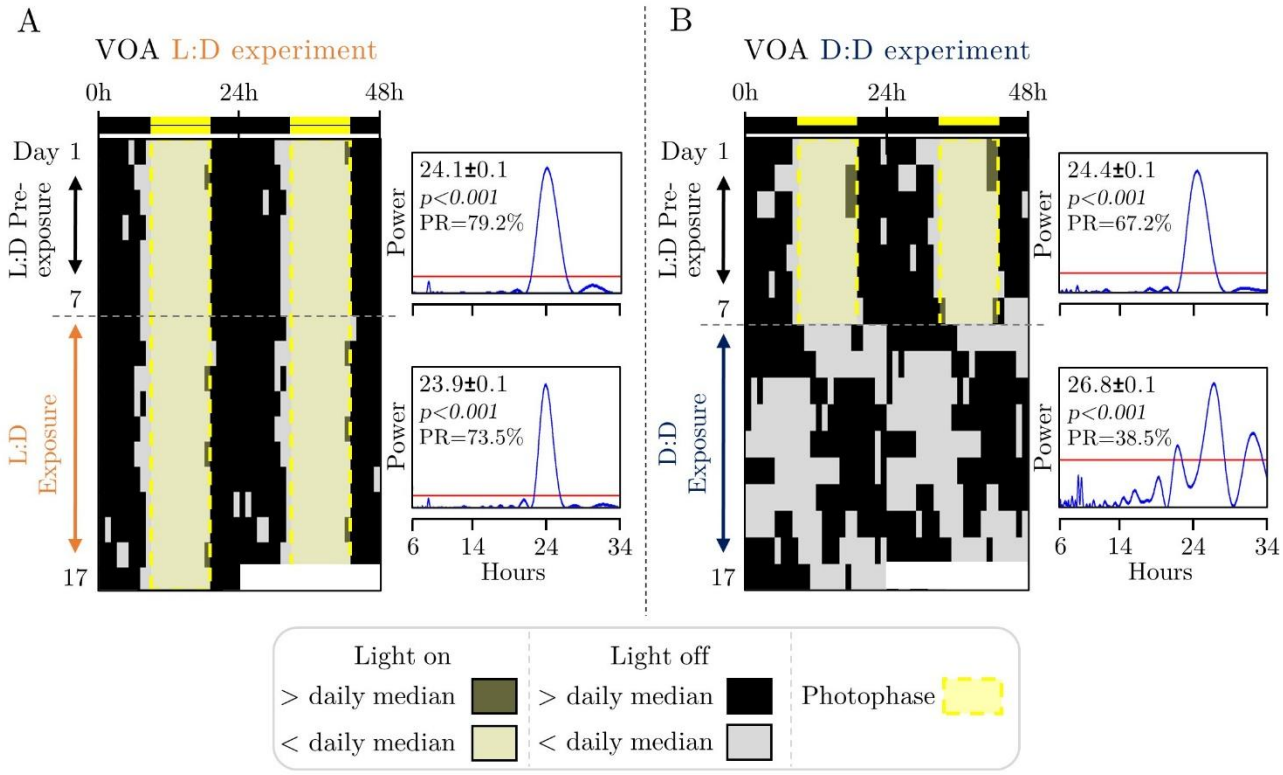


Figure 4.4. Actograms and chronobiological analyses of *O. edulis* valve activity. Actograms of mean valve opening amplitude (VOA, n = 24 individuals per experiment) during the 17 days of the L:D experiment (A) and during the 17 days of the D:D experiment (B). Each actogram represents the 7 days of pre-exposure and the 10 days of exposure for both experiments. For each pre-exposure and exposure phase, the Lomb

& Scargle periodogram (LS) and characteristics of the Cosinor model are associated: the period (h) ± SE (indicated in bold), the *p-value*, and the percent rhythm (PR, %). The red line on the LS periodogram indicates a threshold of significance ($p = 0.95$). The rhythm is considered significant for the Cosinor model with $p < 0.05$. In the actograms, yellow areas indicate the photophase, and the dotted yellow lines represent the times when the light is turned on and off. The yellow and black bars at the top of the actograms depict the light and dark phases, respectively. Hourly individual VOA data are accessible in the electronic supplementary material, dataset S2 (available with the original publication).

Figure 4.4 illustrates the actograms of the mean valve opening amplitude (VOA) during two 17-day experiments and the corresponding results of chronobiological analyses based on the oysters from the two EUs (n = 24 individuals per experiment). After a 7-day pre-exposure (12 L:12 D), two light regimes were tested, each for 10 days: 12 L:12 D (L:D experiment) and D:D (D:D experiment) (Hourly individual VOA data are accessible in the electronic supplementary material, dataset S2 available with the original publication). During the pre-exposure to the 12 L:12 D regime, oysters exhibited a significant daily rhythm of 24.1 ± 0.02 h and 24.4 ± 0.02 h in the L:D experiment and D:D experiment respectively, with a rhythm robustness percentage (PR) averaging 73.2 %. In the following L:D exposure of the L:D experiment, VOA showed a significant daily rhythm of about 23.9 ± 0.02 h, with a PR of 73.5 %. This rhythmicity is comparable to that observed during the pre-exposure with the same 12 L:12 D regime. In the D:D experiment, used to test the endogenous circadian behavioral rhythm, *O. edulis* demonstrated under D:D exposure a persistent rhythm within the circadian range (20 – 28 h), of approximately 26.8 ± 0.02 h, with an associated PR of 38.5 %.

Gene expression in gills and labial palps

Table 4.1. Detailed results of chronobiological analysis in expression levels of clock genes in *Ostrea edulis* gills and labial palps. Results of the RAIN analysis are shown for both light:dark (L:D) and constant darkness (D:D) experiments. For each gene and condition, the detected rhythm period (Per.) is indicated when significant rhythmicity was found in the daily/circadian (20–28 h) or ultradian (10–14 h) ranges. Significant *p*-values ($p < 0.05$) are indicated in bold, and the peak time of maximal expression is reported in ZT/CT. ZT: zeitgeber time; CT: circadian time.

	Genes	Circadian range (20-28h)			Ultradian range (10-14h)			Circadian range (20-28h)			Ultradian range (10-14h)		
		Per.	*p-value*	Peak	Per.	*p-value*	Peak	Per.	*p-value*	Peak	Per.	*p-value*	Peak
		Gills						Labial palps					
L:D	*OeClock*	ns	0.191	ns	14	**0.009**	ZT19	ns	0.072	ns	ns	0.070	ns
	OeBmal1	20	**0.044**	ZT17	ns	0.276	ns	20	**0.046**	ZT17	14	**0.040**	ZT17
	OePer1	ns	0.090	ns	ns	0.056	ns	22	**0.038**	ZT21	ns	0.127	ns
	OeTim1	ns	0.107	ns	ns	0.055	ns	22	**0.038**	ZT5	ns	0.127	ns
	OeCry2	ns	0.221	ns	ns	0.075	ns	22	**0.036**	ZT5	ns	0.127	ns
	OeRev-erb	ns	0.849	ns	ns	0.099	ns	22	**0.017**	ZT11	ns	0.158	ns
	OeRor	20	**0.003**	ZT17	14	**0.012**	ZT17	ns	0.053	ns	14	**0.017**	ZT11
	OeDbt	ns	0.778	ns	10	**0.035**	ZT19	22	**0.046**	ZT5	14	**0.040**	ZT5
	OeCwo	26	**0.010**	ZT17	ns	0.751	ns	ns	0.079	ns	14	**0.040**	ZT17
	OeShag	22	**0.002**	ZT19	ns	0.761	ns	ns	0.064	ns	14	**0.022**	ZT5
	OeHiomt	ns	0.289	ns	ns	0.149	ns	ns	0.146	ns	ns	0.076	ns
	OeCry1	ns	0.174	ns	14	**0.033**	ZT21	ns	0.088	ns	14	**0.017**	ZT5
	OeOpn4	20	**0.000**	ZT1	ns	0.451	ns	22	**0.046**	ZT7	14	**0.040**	ZT7
D:D	*OeClock*	ns	0.425	ns	12	**0.024**	CT5	ns	0.084	ns	ns	0.059	ns
	OeBmal1	ns	0.343	ns	ns	0.124	ns	ns	0.088	ns	14	**0.017**	CT13
	OePer1	ns	0.259	ns	ns	0.072	ns	ns	0.093	ns	ns	0.127	ns
	OeTim1	ns	0.171	ns	12	**0.028**	CT5	ns	0.064	ns	14	**0.022**	CT23
	OeCry2	ns	0.425	ns	ns	0.255	ns	22	**0.038**	CT13	14	**0.010**	CT13
	OeRev-erb	ns	0.068	ns	14	**0.037**	CT13	ns	0.064	ns	14	**0.022**	CT23
	OeRor	ns	0.845	ns	ns	0.060	ns	22	**0.046**	CT23	14	**0.040**	CT23
	OeDbt	ns	0.425	ns	ns	0.348	ns	ns	0.064	ns	14	**0.022**	CT13
	OeCwo	ns	0.198	ns	ns	0.341	ns	20	**0.046**	CT13	14	**0.040**	CT13
	OeShag	ns	0.109	ns	10	**0.000**	CT13	20	**0.017**	CT3	14	**0.031**	CT3
	OeHiomt	ns	0.069	ns	ns	0.401	ns	22	**0.049**	CT13	14	**0.035**	CT13
	OeCry1	ns	0.828	ns	12	**0.031**	CT13	22	**0.036**	CT3	ns	0.070	ns
	OeOpn4	28	**0.014**	CT1	ns	0.232	ns	20	**0.027**	CT1	ns	0.076	ns

Table 4.1 presents the results of RAIN analysis used to assess rhythmicity in the expression of the putative core circadian clock genes (*OeClock*, *OeBmal*, *OePer1*, *OeTim1*, *OeCry2*), genes involved in secondary loops of the circadian clock (*OeRev-erb*, *OeRor*, *OeCwo*), as well as genes involved in post-translational regulations of the circadian clockwork (*OeDbt*, *OeShag*), a gene involved in melatonin synthesis (*OeHiomt*), and photoreceptor genes (*OeCry1* and *OeOpn4*) in the gills and labial palps of *O. edulis* under L:D and D:D conditions (Representative temporal expression profiles are provided in Supplementary Figure S9.8 and Supplementary dataset S3 (available with the original publication) provides detailed gene expression data in gills and labial palps during L:D and D:D experiments). Results presented in the in Supplementary Figure S9.8 showed that all the studied genes are expressed in both tissues. Rhythm analysis conducted using RAIN algorithm revealed daily/circadian (24 ± 4 h) and ultradian (12 ± 2 h) period ranges of oscillations in gene expression for both gills and

labial palps (Tab. 1). Firstly, in the gills under L:D conditions, five genes exhibited significant daily rhythmicity, including *OeBmal1*, *OeRor*, *OeCwo*, *OeShag*, and *OeOpn4*; while *OeClock*, *OeTim1*, *OeCry2,* and *OeCry1* displayed significant ultradian rhythmicity; and *OePer1*, *OeRev-erb*, *OeDbt* and *OeHiomt* showed no significant rhythmicity (Tab. 1). The expression profiles of *OeBmal1*, *OeTim1*, *OeCry2*, *OeRor*, *OeDbt*, *OeCwo* and *OeCry1* showed a nocturnal peak (ZT17, ZT19 or ZT21) while *OeOpn4* displayed two peaks, one at the beginning of the day (ZT1) and another in the middle of the day (ZT7) (Fig. S3). Under D:D conditions, only *OeOpn4* maintained a rhythm in the circadian range, and ultradian rhythmicity was observed for *OeClock*, *OeCry2*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeShag,* and *OeCry1* (Tab. 1). *OeOpn4* only maintained the peak of expression at the beginning of the subjective day (CT1) (Fig. S3). For *OeBmal1*, *OePer1*, *OeRev-erb, OeRor*, *OeDbt*, *OeCwo, OeShag* and *OeCry1,* the nocturnal peak shifted to the subjective early night (CT13) (Fig. S3). Secondly, in the labial palps, rhythmic expression was more pronounced than in the gills under both L:D and D:D regimes. Under L:D condition, significant daily rhythms were detected for seven genes: *OeBmal1*, *OePer1*, *OeTim1*, *OeCry2*, *OeRev-erb*, *OeDbt*, and *OeOpn4*, with three of them (*OeBmal1, OeDbt*, *OeOpn4)* also showing ultradian rhythm, while *OeRor*, *OeCwo*, *OeShag* and *OeCry1* showed only ultradian expression, and *OeClock* and *OeHiomt* showed no significant rhythm (Tab. 1). Notably, *OeOpn4* exhibited comparable profiles than in the gills (Fig. S3). Under D:D conditions, seven genes exhibited a circadian rhythm in labial palps: *OeCry2*, *OeRor*, *OeCwo*, *OeShag*, *OeHiomt*, *OeCry1,* and *OeOpn4*, while nine genes showed ultradian rhythms: *OeBmal1*, *OeTim1*, *OeCry2*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeCwo*, *OeShag*, and *OeHiomt,* including five genes (*OeCry2, OeRor*, *OeCwo*, *OeShag*, *OeHiomt)* with both circadian and ultradian rhythms (Tab. 1). A peak in gene expression was observed during the subjective early night (CT13) for *OeBmal1*, *OePer1*, *OeCry2*, *OeDbt*, *OeCwo*, *OeShag*, *OeHiomt,* and *OeCry1,* while *OeOpn4*showed peaks during the subjective day (CT1) (Fig. S3).

Discussion

In this study, data mining and protein domain analysis identified 13 key components putatively involved in the circadian clock functioning of the flat oyster *Ostrea edulis.* These include orthologs of circadian clock genes, light reception genes, and a gene related to melatonin synthesis. Phylogenetic analyses revealed that the identified gene sequences shared strong homology with genes described in circadian clock models from other species, indicating conservation across diverse taxa. Notably, our findings suggest the

presence of two distinct types of cryptochromes in *O. edulis*: *OeCry2*, which would be directly implicated in the main circadian loop, and *OeCry1*, which would function as a circadian photoreceptor. At the molecular level, rhythmic mRNA expression patterns were observed in gill and labial palp tissues, with some genes exhibiting daily and/or ultradian rhythms under light:dark (L:D) and constant dark (D:D) conditions, suggesting a plastic and self-sustained mechanism. Although endogenous clocks have been described in some marine invertebrates (Biscontin et al., 2017; Zantke et al., 2013), including a few bivalves (Perrigault & Tran, 2017), their functional characterization in oysters remains limited. Our findings in *O. edulis* expand the phylogenetic range of bivalves and demonstrate endogenous circadian regulation of valve behavior with synchronized daily valve activity under L:D conditions, as well as a maintained circadian rhythm in free-running conditions (D:D). These behavioral rhythms, observed as an output of the molecular clock, provide strong evidence for a functional endogenous clock mechanism in this species.

Circadian clock genes in O. edulis.

This study identified five genes in O. edulis as orthologs of genes involved in the core loop of the circadian clock in other species: OeClock, OeBmal1, OePer1, OeTim1, and OeCry2. These genes are conserved across major metazoan lineages, including vertebrates (Deuterostomia), Ecdysozoans such as D. melanogaster (Stanton et al., 2022) and Euphausia superba (Biscontin et al., 2017), and Lophotrochozoans such as P. dumerilii (Zantke et al., 2013) and C. gigas (Perrigault & Tran, 2017). They form positive and negative feedback loops, producing a self-sustained oscillation for approximately 24-hour period, regulated by environmental cues (Cermakian & Sassone-Corsi, 2000; Partch et al., 2014). The presence of these genes suggests a similar function of O. edulis's circadian core clock. Additionally, OeRor and OeRev-erb were recognized as orthologs of ror and rev-erb known to regulate Bmal1 transcription and enhance clock robustness (Jetten & Joo, 2006; E. E. Zhang & Kay, 2010). In vertebrates and phylogenetically close species such as C. gigas, rev-erb has been described as an inhibitor, while ror acts as an activator of Bmal1 expression (Guillaumond et al., 2005; Relógio et al., 2011). We also identified additional genes, orthologs of clock genes known in Drosophila to be involved in secondary loop of the circadian clock (*Cwo*), or genes involved in post-translational regulations of the circadian clockwork (*Dbt* and Shag) (Matsumoto et al., 2007; Nawathean & Rosbash, 2004). Though less studied in marine organisms, these genes have been identified in oyster species such as Crassostrea angulata

for *shaggy* (Zeng et al., 2013) as well as in the copepod Calanus finmarchicus or the scallop Pecten yessoensis for *doubletime* (Hüppe et al., 2020; Sun et al., 2016). Their identification in *O. edulis* could indicate their crucial role and implication in the molecular clock regulation mechanism.

We identified two types of cryptochromes in O. edulis, OeCry1 and OeCry2, both possessing conserved protein domains characteristic of photolyase/CRY family proteins (Chaves et al., 2011; Öztürk et al., 2007). Cryptochromes are classified by their function; the first type, *OeCry1*, is referred to as "*Drosophila*-type" cryptochromes, and serves as blue-light-sensitive photoreceptors. They provide environmental input to the core clock by interacting with the TIM1-PER heterodimer in a light-dependent manner promoting light-induced degradation of TIM1 (Emery et al., 1998, 2000; Lin et al., 2001; Peschel et al., 2009; Stanewsky et al., 1998). In contrast, the second type, *Oe*Cry2, is referred to as "Mammalian-type" cryptochromes and acts as non-photosensitive repressors that inhibit CLOCK/BMAL1 activity (Yuan et al., 2007; Zhu et al., 2005). Phylogenetic analysis grouped OeCry1 with the "*Drosophila*-type" cryptochromes and OeCry2 with the "Mammalian-type" cryptochromes. The identification of the phylogenetic trees of these two cryptochrome types, supports “butterfly-type” molecular mechanisms in *O. edulis* described in the monarch butterfly Danaus plexippus (Zhu et al., 2005), as suggested in the marine copepod *C. finmarchicus* (Christie et al., 2013; Häfker et al., 2017) or the oyster C. gigas (Perrigault & Tran, 2017), where the principal positive feedback loop involves the CLOCK/BMAL1 heterodimer, and the primary negative feedback loop involves the TIM1/PER/CRY2 complex, with CR1 interacting with TIM1 for photo-entrainment.

The study also allowed for identification of another putative light reception gene, OeOpn4, an ortholog of *opsin4* (*melanopsin*), which is involved in the detection of light intensity (Bellingham et al., 2002; Hankins et al., 2008; Peirson & Foster, 2006), highlighting the potential of opsins for exploring the photoreceptive repertoire contributing to circadian regulation in *O.edulis*. The identification of this gene, along with *OeCry1*, which is known to be involved in the detection of short wavelengths of light, in the blue range, suggests that blue light could play a role in the synchronization of the endogenous clock through non-visual photoreceptors in O. edulis. Although direct evidence for wavelength sensitivity in *O. edulis* is lacking, previous studies in bivalves (Botté et al., 2023; M. Zhang et al., 2023) indicate light-mediated behavioral rhythms

and putative photoreceptors, supporting a potential role of light perception in circadian regulation.

Finally, we identified OeHiomt, which is predicted to encode hydroxyindole-O-methyltransferase (HIOMT), an enzyme involved in melatonin (N-acetyl-5-methoxytryptamine) synthesis (Foulkes et al., 1997). Melatonin is an evolutionarily conserved hormone implicated in regulating temporal rhythms across daily, lunar, and annual cycles in various invertebrates, vertebrates, plants, and bacteria (Ikegami & Yoshimura, 2017; Reiter, 1993; Tosches et al., 2014). The presence of *OeHiomt* highlights the potential role of melatonin in the circadian machinery in O. edulis.

Overall, the identification of the circadian clock molecular components and validation of conserved protein domains indicate that the endogenous clock in *O. edulis* may share a similar architecture with phylogenetically close species such as *C. gigas*. To go further, we investigated the functionality of the circadian clock in *O. edulis*.

Investigation of the functionality of the circadian clock

We then investigated whether the identified circadian clock genes are expressed in two different tissues (gills and labial palps) of O. edulis, and if they are rhythmically expressed. We also investigated the temporal expression level of all the other genes identified in this study, putatively under clock control, and we concomitantly studied the temporal valve behavior patterns of *O. edulis*, also putatively under clock control. Oysters valve opening is required to filter water, allowing oxygen supply and nutritional physiological processes in which gills and labial palps are actively involved. Gills have a role in respiration but also in nutrition as they permit the transport of food particles to the labial palps, while labial palps manage the final selection and transfer of food particles to the digestive tract (Fabra et al., 2024).

The observed rhythmic gene expression and behavior support the existence of a functional endogenous oscillator. Interestingly, while valve activity showed clear daily (under L:D) and circadian (under D:D) rhythms as described in a previous study (Le Moal et al., 2025), the temporal expression patterns of both circadian clock gene and putative clock-controlled genes were more variable, showing some daily/circadian rythms but also ultradian ones, as well as bimodal (both daily/circadian and ultradian periodicities) and arythmic patterns, depending on genes and tissues. This discrepancy between behavioral and molecular rhythms, and between the two tissues, may be attributed to tissue-specific oscillations (Mure et al., 2018). It may be suggested that the

adductor muscle, directly involved in the valve activity, expresses a different profile of clock gene oscillations from those observed in gills and labial palps. Furthermore, the tissue-specific physiological functions of gills and labial palps may require distinct temporal organization, explaining differences in expression profiles observed in both core circadian clock genes and putative clock-controlled genes. Additionally, it has to be noted that the molecular approach is more sensitive to individual variability than the behavioral approach due to methodological reasons. Time series sampling for molecular investigation requires sacrificing individuals at each time point. Furthermore, both the number of oysters per time point (8 individuals) and the sampling frequency are limited by logistical reasons. In contrast, the behavioral analysis, based on the continuous recording of the same 24 individuals throughout the whole experiment, is more efficient in revealing a rhythmic pattern. Nevertheless, despite individual variability, the different rhythmicities (daily/circadian, ultradian, or bimodal) observed at the molecular level, may reflect an intrinsic plasticity and or lability of the circadian clock's functioning in *O.edulis*.

A plastic and labile clock in O. edulis

The clock plasticity observed in this study at the molecular level is in accordance with a previous study where the circadian behavior of *O. edulis* was described as labile and plastic (Le Moal et al., 2025). This plasticity may reflect an adaptive feature aligned with Enright's hypothesis of a single, flexible oscillator governing multiple temporal rhythms in marine organisms, allowing adaptation to both daily and tidal environmental cycles (Enright, 1976). This hypothesis is supported by studies of species like the oyster *C. gigas* (Mat et al., 2016; Payton et al., 2017; Tran et al., 2020) or the copepod *C. finmarchicus* (Hüppe et al., 2020). In our study, and in absence of tidal entrainment, the ultradian rhythms could be interpreted as circatidal rhythms, revealing the putative endogenous capacity of clock components to be entrained by tidal environmental cues. The oysters used in this study used to be cultivated in natural subtidal conditions, however it does not exclude that they may have been exposed to tidal forces other than emersion. As observed in *C. gigas*, bimodal rhythms of circadian clock genes expression are also expressed, i.e. a mix between both daily/circadian and tidal periodicities (Tran et al., 2020). However, unlike what is observed under pure L:D entrainement in *C. gigas* (Tran et al., 2020), a relatively high number of circadian genes are found to show ultradian rhythms under the same condtions in *O. edulis*. Further studies combining both daily and tidal *zeitgebers* would be necessary to better understand and characterize the molecular clock functioning and entrainment in *O. edulis*. In the field, the clock

plasticity may confer advantages, allowing marine organisms to adapt to their complex biotope, which is subjected to multiple cycles (Naylor, 2010). It could also be advantageous for adaptation to temperature shifts due to global warming, as discussed in (Le Moal et al., 2025). However, it could also increase sensitivity to anthropogenic pressures such as artificial light at night, potentially influencing coastal populations of *O. edulis*. Understanding this vulnerability is crucial for conservation efforts to enhance the species' resilience to environmental changes.

Conclusion

This study identified the orthologs of key circadian clock components in *Ostrea edulis*, highlighting the conserved structural and functional domains of their protein sequences. Expression profiling of these clock genes has provided new insights into the molecular mechanisms underlying clock-driven phenotypes in this bivalve. Understanding the molecular basis of these biological rhythms is essential for enhancing our comprehension of this European native oyster, as well as for better understanding its response to environmental cycles and improving conservation strategies for the species. The labile aspect of the clock discussed in this study may facilitate further adaptation to temperature shifts due to global warming. On the contrary, the labile element of the clock may also reinforce negative aspects of anthropogenic activity, such as the expansion of artificial light at night. In this perspective, our findings present a first approach. They will facilitate further investigation of circadian clock genes in *O. edulis,* providing genetic resources for exploring the genetic mechanisms of this biological clock. However, to fully characterize the molecular clock mechanism in *O. edulis*, further functional approaches, both in laboratory and natural conditions, will be necessary to better define the relationships between these genes and their specific roles within the clock mechanism in *O. edulis*.

Acknowledgments

A. Le Moal was supported by a grant awarded to B. Meyer and B. Pogoda via AWI Inspires - International Science Program for Integrative Research in Earth Systems. The study contributes to the Helmholtz Research Program "Changing Earth-Sustaining our future" of the research field Earth and Environment of the Helmholtz Association, Topic 6, Subtopic 6.1. and 6.2 as well as Topic 4, Suptopic 4.2. The project was supported by the "PHC PROCOPE" program via the project "OysterClock" (project-ID: 57701739),

funded by the French Ministry for Europe and Foreign Affairs, the French Ministry for Higher Education and Research and the German Academic Exchange Service (DAAD). The was also supported by the French National Research Agency (ANR), LUCIOLE project (ANR-22-CE34- 0010-01). We thank Yannick Geerebaert for his help during the setup of the experiment. We thank Christophe Klopp for his help on the bio-informatic and data mining work. We thank Laure Bellec for her help on the phylogenetic work. We thank Cerise Daffe for the help during the design of the primers. We thank Kristin Tessmar-Raible and Stephane Pouvreau for their valuable discussions.

Data availability

The sequences of genes used to construct phylogenetic trees can be found via accession numbers given in the electronic supplementary material, table S9.2 for *Ostrea edulis* and dataset S1 (available with the original publication) for other species. Hourly individual VOA data from L:D and D:D laboratory experiments are accessible in the electronic supplementary material, dataset S2 (available with the original publication). Gene expression data in gills and labial palps during L:D and D:D laboratory experiments are accessible in the electronic supplementary material, dataset S3 (available with the original publication). Supplementary table S9.3 provides detailed results of RAIN analysis in expression levels of clock genes in *Ostrea edulis* gills and labial palps.

References

Adema, C. M. (2021). Sticky problems : Extraction of nucleic acids from molluscs. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1825), 20200162. https://doi.org/10.1098/rstb.2020.0162

Andreatta, G., & Tessmar-Raible, K. (2020). The Still Dark Side of the Moon : Molecular Mechanisms of Lunar-Controlled Rhythms and Clocks. *Journal of Molecular Biology, Circadian Regulation: from Molecules to Physiology*, *432*(12), 3525-3546. https://doi.org/10.1016/j.jmb.2020.03.009

Bellingham, J., Whitmore, D., Philp, A. R., Wells, D. J., & Foster, R. G. (2002). Zebrafish melanopsin : Isolation, tissue localisation and phylogenetic position. *Molecular Brain Research*, *107*(2), 128-136. https://doi.org/10.1016/S0169-328X(02)00454-0

Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators : Lessons from diverse organisms. *Nature Reviews Genetics*, *6*(7), 544-556. https://doi.org/10.1038/nrg1633

Bingham, C., Arbogast, B., Guillaume, G. C., Lee, J. K., & Halberg, F. (1982). Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*, *9*(4), 397-439.

Biscontin, A., Wallach, T., Sales, G., Grudziecki, A., Janke, L., Sartori, E., Bertolucci, C., Mazzotta, G., De Pittà, C., Meyer, B., Kramer, A., & Costa, R. (2017). Functional characterization of the circadian clock in the Antarctic krill, Euphausia superba. *Scientific Reports*, *7*(1), 17742. https://doi.org/10.1038/s41598-017-18009-2

Botté, A., Payton, L., & Tran, D. (2023). The effects of artificial light at night on behavioral rhythm and related gene expression are wavelength dependent in the oyster Crassostrea gigas. *Environmental Science and Pollution Research*, *30*(57), 120375-120386. https://doi.org/10.1007/s11356-023-30793-1

Box, G. E. P., Jenkins, G. M., & Reinsel, G. C. (1994). *Time series analysis : Forecasting and control* (Third edition). Prentice Hall. http://www.gbv.de/dms/bowker/toc/9780130607744.pdf

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines : Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, *55*(4), 611-622. https://doi.org/10.1373/clinchem.2008.112797

Cermakian, N., & Sassone-Corsi, P. (2000). Multilevel regulation of the circadian clock. *Nature Reviews Molecular Cell Biology*, *1*(1), 59-67. https://doi.org/10.1038/35036078

Chapman, E. C., O'Dell, A. R., Meligi, N. M., Parsons, D. R., & Rotchell, J. M. (2017). Seasonal expression patterns of clock-associated genes in the blue mussel Mytilus edulis. *Chronobiology International*, *34*(9), 1300-1314. https://doi.org/10.1080/07420528.2017.1363224

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.-O., Horst, G. T. J. van der, Batschauer, A., & Ahmad, M. (2011). The Cryptochromes : Blue Light Photoreceptors in Plants and Animals. *Annual Review of Plant Biology*, *62*(Volume 62, 2011), 335-364. https://doi.org/10.1146/annurev-arplant-042110-103759

Christie, A. E., Fontanilla, T. M., Nesbit, K. T., & Lenz, P. H. (2013). Prediction of the protein components of a putative *Calanus finmarchicus* (Crustacea, Copepoda) circadian signaling system using a *de novo* assembled transcriptome. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *8*(3), 165-193. https://doi.org/10.1016/j.cbd.2013.04.002

Colsoul, B., Boudry, P., Pérez-Parallé, M. L., Bratoš Cetinić, A., Hugh-Jones, T., Arzul, I., Mérou, N., Wegner, K. M., Peter, C., Merk, V., & Pogoda, B. (2021). Sustainable large-scale production of European flat oyster (Ostrea edulis) seed for ecological restoration and aquaculture : A review. *Reviews in Aquaculture*, *13*(3), 1423-1468. https://doi.org/10.1111/raq.12529

Davison, A., & Neiman, M. (2021). Mobilizing molluscan models and genomes in biology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1825), 20200163. https://doi.org/10.1098/rstb.2020.0163

Du, Y., Zhang, L., Xu, F., Huang, B., Zhang, G., & Li, L. (2013). Validation of housekeeping genes as internal controls for studying gene expression during Pacific oyster (*Crassostrea gigas*) development by quantitative real-time PCR. *Fish & Shellfish Immunology*, *34*(3), 939-945. https://doi.org/10.1016/j.fsi.2012.12.007

Dubruille, R., & Emery, P. (2008). A Plastic Clock : How Circadian Rhythms Respond to Environmental Cues in Drosophila. *Molecular Neurobiology*, *38*(2), 129-145. https://doi.org/10.1007/s12035-008-8035-y

Dunlap, J. C. (1999). Molecular Bases for Circadian Clocks. *Cell*, *96*(2), 271-290. https://doi.org/10.1016/S0092-8674(00)80566-8

Emery, P., So, W. V., Kaneko, M., Hall, J. C., & Rosbash, M. (1998). CRY, a Drosophila Clock and Light-Regulated Cryptochrome, Is a Major Contributor to Circadian Rhythm Resetting and Photosensitivity. *Cell*, *95*(5), 669-679. https://doi.org/10.1016/S0092-8674(00)81637-2

Emery, P., Stanewsky, R., Helfrich-Förster, C., Emery-Le, M., Hall, J. C., & Rosbash, M. (2000). Drosophila CRY Is a Deep Brain Circadian Photoreceptor. *Neuron*, *26*(2), 493-504. https://doi.org/10.1016/S0896-6273(00)81181-2

Enright, J. T. (1976). Plasticity in an isopod's clockworks : Shaking shapes form and affects phase and frequency. *Journal of Comparative Physiology*, *107*(1), 13-37. https://doi.org/10.1007/BF00663916

Fabra, M., Morrall, Z., Helmer, L., Watson, G., & Preston, J. (2024). Filtration Behaviour of Ostrea edulis : Diurnal Rhythmicity Influenced by Light Cycles, Body Size and Water Temperature. *Estuaries and Coasts*, *48*(1), 18. https://doi.org/10.1007/s12237-024-01453-5

Foulkes, N. S., assone-Corsi, P., Borjigin, J., & Snyder, S. H. (1997). Rhythmic transcription : The molecular basis of circadian melatonin synthesis. *Trends in Neurosciences*, *20*(10), 487-492. https://doi.org/10.1016/S0166-2236(97)01109-0

Goto, S. G. (2013). Roles of circadian clock genes in insect photoperiodism. *Entomological Science*, *16*(1), 1-16. https://doi.org/10.1111/ens.12000

Guillaumond, F., Dardente, H., Giguère, V., & Cermakian, N. (2005). Differential Control of Bmal1 Circadian Transcription by REV-ERB and ROR nuclear Receptors. *Journal of Biological Rhythms*, *20*(5), 391-403. https://doi.org/10.1177/0748730405277232

Gundappa, M. K., Peñaloza, C., Regan, T., Boutet, I., Tanguy, A., Houston, R. D., Bean, T. P., & Macqueen, D. J. (2022). Chromosome-level reference genome for European flat oyster (Ostrea edulis L.). *Evolutionary Applications*, *15*(11), 1713-1729. https://doi.org/10.1111/eva.13460

Haelters, J., & Kerckhof, F. (2009). *Background document for Ostrea edulis and Ostrea edulis bed*. OSPAR Commission – Biodiversity Series, pp. 1–22. Anonymous, London.

Häfker, N. S., Meyer, B., Last, K. S., Pond, D. W., Hüppe, L., & Teschke, M. (2017). Circadian Clock Involvement in Zooplankton Diel Vertical Migration. *Current Biology*, *27*(14), 2194-2201.e3. https://doi.org/10.1016/j.cub.2017.06.025

Hankins, M. W., Peirson, S. N., & Foster, R. G. (2008). Melanopsin : An exciting photopigment. *Trends in Neurosciences*, *31*(1), 27-36. https://doi.org/10.1016/j.tins.2007.11.002

Hardin, P. E. (2005). The Circadian Timekeeping System of Drosophila. *Current Biology*, *15*(17), R714-R722. https://doi.org/10.1016/j.cub.2005.08.019

Herrero, M. J., & Lepesant, J. M. J. (2014). Daily and seasonal expression of clock genes in the pituitary of the European sea bass (*Dicentrarchus labrax*). *General and Comparative Endocrinology*, *208*, 30-38. https://doi.org/10.1016/j.ygcen.2014.08.002

Hüppe, L., Payton, L., Last, K., Wilcockson, D., Ershova, E., & Meyer, B. (2020). Evidence for oscillating circadian clock genes in the copepod Calanus finmarchicus during the summer solstice in the high Arctic. *Biology Letters*, *16*(7), 20200257. https://doi.org/10.1098/rsbl.2020.0257

Ikegami, K., & Yoshimura, T. (2017). Molecular Mechanism Regulating Seasonality. In V. Kumar (Éd.), *Biological Timekeeping : Clocks, Rhythms and Behaviour* (p. 589-605). Springer India. https://doi.org/10.1007/978-81-322-3688-7_28

Jetten, A. M., & Joo, J. H. (2006). Retinoid-related orphan receptors (RORs) : Roles in cellular differentiation and development. In *Advances in Developmental Biology* (Vol. 16, p. 313-355). Elsevier. https://doi.org/10.1016/S1574-3349(06)16010-X

Le Moal, A., Payton, L., Andrade, H., Camus, L., Ballantine, C., Ciret, P., & Tran, D. (2023). Blue mussels' valve behavior exhibits daily and lunar rhythms during the high Arctic polar day. *Marine Biology*, *170*(9), 113. https://doi.org/10.1007/s00227-023-04257-6

Le Moal, A., Tran, D., Payton, L., Pogoda, B., & Meyer, B. (2025). Characterization of the daily and circadian valve behavior of the European flat oyster Ostrea edulis. *Scientific Reports*, *15*(1), 14952. https://doi.org/10.1038/s41598-025-98746-x

Lin, F.-J., Song, W., Meyer-Bernstein, E., Naidoo, N., & Sehgal, A. (2001). Photic Signaling by Cryptochrome in the Drosophila Circadian System. *Molecular and Cellular Biology*, *21*(21), 7287-7294. https://doi.org/10.1128/MCB.21.21.7287-7294.2001

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, *25*(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Mat, A. M., Perrigault, M., Massabuau, J.-C., & Tran, D. (2016). Role and expression of cry1 in the adductor muscle of the oyster Crassostrea gigas during daily and tidal valve activity rhythms. *Chronobiology International*, *33*(8), 949-963. https://doi.org/10.1080/07420528.2016.1181645

Matsumoto, A., Ukai-Tadenuma, M., Yamada, R. G., Houl, J., Uno, K. D., Kasukawa, T., Dauwalder, B., Itoh, T. Q., Takahashi, K., Ueda, R., Hardin, P. E., Tanimura, T., & Ueda, H. R. (2007). A functional genomics strategy reveals clockwork orange as a transcriptional regulator in the Drosophila circadian clock. *Genes & Development*, *21*(13), 1687-1700. https://doi.org/10.1101/gad.1552207

Morga, B., Arzul, I., Faury, N., & Renault, T. (2010). Identification of genes from flat oyster *Ostrea edulis* as suitable housekeeping genes for quantitative real time PCR. *Fish & Shellfish Immunology*, *29*(6), 937-945. https://doi.org/10.1016/j.fsi.2010.07.028

Mure, L. S., Le, H. D., Benegiamo, G., Chang, M. W., Rios, L., Jillani, N., Ngotho, M., Kariuki, T., Dkhissi-Benyahya, O., Cooper, H. M., & Panda, S. (2018). Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. *Science*, *359*(6381), eaao0318. https://doi.org/10.1126/science.aao0318

Nangle, S. N., Rosensweig, C., Koike, N., Tei, H., Takahashi, J. S., Green, C. B., & Zheng, N. (2014). Molecular assembly of the period-cryptochrome circadian transcriptional repressor complex. *eLife*, *3*, e03674. https://doi.org/10.7554/eLife.03674

Nawathean, P., & Rosbash, M. (2004). The Doubletime and CKII Kinases Collaborate to Potentiate Drosophila PER Transcriptional Repressor Activity. *Molecular Cell*, *13*(2), 213-223. https://doi.org/10.1016/S1097-2765(03)00503-3

Naylor, E. (2010). *Chronobiology of Marine Organisms*. Cambridge University Press. https://doi.org/10.1017/CBO9780511803567

Öztürk, N., Song, S.-H., Özgür, S., Selby, C. P., Morrison, L., Partch, C., Zhong, D., & Sancar, A. (2007). Structure and Function of Animal Cryptochromes. *Cold Spring Harbor Symposia on Quantitative Biology*, *72*, 119-131. https://doi.org/10.1101/sqb.2007.72.015

Palmer, J. D. (1995). Review of the Dual-Clock Control of Tidal Rhythms and the Hypothesis that the Same Clock Governs Both Circatidal and Circadian Rhythms. *Chronobiology International*, *12*(5), 299-310. https://doi.org/10.3109/07420529509057279

Partch, C. L., Green, C. B., & Takahashi, J. S. (2014). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology*, *24*(2), 90-99. https://doi.org/10.1016/j.tcb.2013.07.002

Payton, L., Perrigault, M., Hoede, C., Massabuau, J.-C., Sow, M., Huvet, A., Boullot, F., Fabioux, C., Hegaret, H., & Tran, D. (2017). Remodeling of the cycling transcriptome of the oyster Crassostrea gigas by the harmful algae Alexandrium minutum. *Scientific Reports*, *7*(1), 3480. https://doi.org/10.1038/s41598-017-03797-4

Peirson, S., & Foster, R. G. (2006). Melanopsin : Another Way of Signaling Light. *Neuron*, *49*(3), 331-339. https://doi.org/10.1016/j.neuron.2006.01.006

Perrigault, M., Andrade, H., Bellec, L., Ballantine, C., Camus, L., & Tran, D. (2020). Rhythms during the polar night : Evidence of clock-gene oscillations in the Arctic scallop Chlamys islandica. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1933), 20201001. https://doi.org/10.1098/rspb.2020.1001

Perrigault, M., & Tran, D. (2017). Identification of the Molecular Clockwork of the Oyster Crassostrea gigas. *PLOS ONE*, *12*(1), e0169790. https://doi.org/10.1371/journal.pone.0169790

Peschel, N., Chen, K. F., Szabo, G., & Stanewsky, R. (2009). Light-Dependent Interactions between the Drosophila Circadian Clock Factors Cryptochrome, Jetlag, and Timeless. *Current Biology*, *19*(3), 241-247. https://doi.org/10.1016/j.cub.2008.12.042

Pogoda, B. (2019). Current Status of European Oyster Decline and Restoration in Germany. *Humanities*, *8*(1), Article 1. https://doi.org/10.3390/h8010009

Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., Sanderson, W., & Nordheim, H. von. (2019). The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation : Bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquatic Living Resources*, *32*, 13. https://doi.org/10.1051/alr/2019012

Pogoda, B., Merk, V., Colsoul, B., Hausen, T., Peter, C., Pesch, R., Kramer, M., Jaklin, S., Holler, P., Bartholomä, A., Michaelis, R., & Prinz, K. (2020). Site selection for biogenic reef restoration in offshore environments : The Natura 2000 area Borkum Reef Ground as a case study for native oyster restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2163-2179. https://doi.org/10.1002/aqc.3405

Pouvreau, S., Lapègue, S., Arzul, I., & Boudry, P. (2023). Fifty years of research to counter the decline of the European flat oyster (Ostrea edulis) : A review of French achievements and prospects for the restoration of remaining beds and revival of aquaculture production. *Aquatic Living Resources*, *36*, 13. https://doi.org/10.1051/alr/2023006

R Core Team. (2023). *R: A language and environment for statistical computing*. [Logiciel]. Foundation for Statistical Computing. URL https://www.R-project.org/

Reiter, R. J. (1993). The melatonin rhythm : Both a clock and a calendar. *Experientia*, *49*(8), 654-664. https://doi.org/10.1007/BF01923947

Relógio, A., Westermark, P. O., Wallach, T., Schellenberg, K., Kramer, A., & Herzel, H. (2011). Tuning the Mammalian Circadian Clock : Robust Synergy of Two Loops. *PLOS Computational Biology*, *7*(12), e1002309. https://doi.org/10.1371/journal.pcbi.1002309

Reppert, S. M., & Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature*, *418*(6901), 935-941. https://doi.org/10.1038/nature00965

Scargle, J. D. (1982). Studies in astronomical time series analysis. II. Statistical aspects of spectral analysis of unevenly spaced data. *The Astrophysical Journal*, *263*, 835-853. https://doi.org/10.1086/160554

Schmalen, I., Reischl, S., Wallach, T., Klemz, R., Grudziecki, A., Prabu, J. R., Benda, C., Kramer, A., & Wolf, E. (2014). Interaction of Circadian Clock Proteins CRY1 and PER2 Is Modulated by Zinc Binding and Disulfide Bond Formation. *Cell*, *157*(5), 1203-1215. https://doi.org/10.1016/j.cell.2014.03.057

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S. A., Rosbash, M., & Hall, J. C. (1998). The cryb Mutation Identifies Cryptochrome as a Circadian Photoreceptor in Drosophila. *Cell*, *95*(5), 681-692. https://doi.org/10.1016/S0092-8674(00)81638-4

Stanton, D., Justin, H. S., & Reitzel, A. M. (2022). Step in Time : Conservation of Circadian Clock Genes in Animal Evolution. *Integrative and Comparative Biology*, *62*(6), 1503-1518. https://doi.org/10.1093/icb/icac140

Sun, X. J., Zhou, L. Q., Tian, J. T., Liu, Z. H., Wu, B., Dong, Y. H., Yang, A. G., & Ma, W. M. (2016). Transcriptome survey of phototransduction and clock genes in marine bivalves. *Genetics and Molecular Research: GMR*, *15*(4). https://doi.org/10.4238/gmr15048726

Thaben, P. F., & Westermark, P. O. (2014). Detecting Rhythms in Time Series with RAIN. *Journal of Biological Rhythms*, *29*(6), 391-400. https://doi.org/10.1177/0748730414553029

Tosches, M. A., Bucher, D., Vopalensky, P., & Arendt, D. (2014). Melatonin Signaling Controls Circadian Swimming Behavior in Marine Zooplankton. *Cell*, *159*(1), 46-57. https://doi.org/10.1016/j.cell.2014.07.042

Tran, D., Andrade, H., Camus, L., Leopold, P., Ballantine, C., Berge, J., Durier, G., Sow, M., & Ciret, P. (2023). Evidence of separate influence of moon and sun on light synchronization of mussel's daily rhythm during the polar night. *iScience*, *26*(3). https://doi.org/10.1016/j.isci.2023.106168

Tran, D., Perrigault, M., Ciret, P., & Payton, L. (2020). Bivalve mollusc circadian clock genes can run at tidal frequency. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192440. https://doi.org/10.1098/rspb.2019.2440

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), research0034.1. https://doi.org/10.1186/gb-2002-3-7-research0034

Yuan, Q., Metterville, D., Briscoe, A. D., & Reppert, S. M. (2007). Insect Cryptochromes : Gene Duplication and Loss Define Diverse Ways to Construct Insect Circadian Clocks. *Molecular Biology and Evolution*, *24*(4), 948-955. https://doi.org/10.1093/molbev/msm011

Zantke, J., Ishikawa-Fujiwara, T., Arboleda, E., Lohs, C., Schipany, K., Hallay, N., Straw, A. D., Todo, T., & Tessmar-Raible, K. (2013). Circadian and Circalunar Clock Interactions in a Marine Annelid. *Cell Reports*, *5*(1), 99-113. https://doi.org/10.1016/j.celrep.2013.08.031

Zeng, Z., Ni, J., & Ke, C. (2013). Expression of glycogen synthase (*GYS*) and glycogen synthase kinase 3β (*GSK3*β) of the Fujian oyster, *Crassostrea angulata*, in relation to glycogen content in gonad development. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *166*(3), 203-214. https://doi.org/10.1016/j.cbpb.2013.09.003

Zhang, E. E., & Kay, S. A. (2010). Clocks not winding down : Unravelling circadian networks. *Nature Reviews Molecular Cell Biology*, *11*(11), 764-776. https://doi.org/10.1038/nrm2995

Zhang, L., Hastings, M. H., Green, E. W., Tauber, E., Sladek, M., Webster, S. G., Kyriacou, C. P., & Wilcockson, D. C. (2013). Dissociation of Circadian and Circatidal Timekeeping in the Marine Crustacean Eurydice pulchra. *Current Biology*, *23*(19), 1863-1873. https://doi.org/10.1016/j.cub.2013.08.038

Zhang, M., Kong, F., Ma, B., Chen, D., Ran, Z., Ma, S., Liao, K., Cao, J., Zhang, L., Yan, X., & Xu, J. (2023). Effects of light on growth, feeding rate, digestion, and antioxidation in juvenile razor clams *Sinonovacula constricta*. *Aquaculture*, *568*, 739306. https://doi.org/10.1016/j.aquaculture.2023.739306

Zhu, H., Yuan, Q., Froy, O., Casselman, A., & Reppert, S. M. (2005). The two CRYs of the butterfly. *Current Biology*, *15*(23), R953-R954. https://doi.org/10.1016/j.cub.2005.11.030

Zu Ermgassen, P. S. E., Thurstan, R. H., Corrales, J., Alleway, H., Carranza, A., Dankers, N., DeAngelis, B., Hancock, B., Kent, F., McLeod, I., Pogoda, B., Liu, Q., & Sanderson, W. G. (2020). The benefits of bivalve reef restoration :

A global synthesis of underrepresented species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2050-2065. https://doi.org/10.1002/aqc.3410

5. Article III - Phenology of *Ostrea edulis* and its environmental drivers

The phenology of the European flat oyster (*Ostrea edulis*) and its environmental drivers

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Published in Scientific reports - https://doi.org/10.1038/s41598-026-63105-x

Abstract

The European flat oyster (*Ostrea edulis*) is an ecosystem engineer that structures reef habitats and is a major focus of restoration efforts across Europe, yet its biological rhythms in its habitat remain unknown. These rhythms synchronize physiology with environmental cycles, indicating ecological adaptation. Here, we investigated behavioral, physiological, and molecular rhythms in *O. edulis*, as well as their environmental drivers. Using high-resolution valvometry, we monitored valve activity and shell growth over 540-days in the German Bight (Helgoland), along with environmental variables such as photoperiod, temperature, underwater sound, and food availability. Monthly gene expression profiling of clock-related genes was also performed. *O. edulis* displayed coordinated daily, tidal, lunar, semilunar, and annual rhythms, mainly driven by photoperiod and temperature. This integrative chronobiological approach reveals a multiple rhythm system in *O. edulis*, highlighting both adaptive capacity and vulnerability to anthropogenic pressures, and providing key indicators for the conservation and restoration of oyster reef habitats.

Keywords: Bivalve shellfish, valve behavior, restoration, shell growth, field experiment, phenology.

Introduction

European flat oyster *Ostrea edulis* (Linnaeus 1758) reef habitats are among the most endangered and degraded marine habitats in Europe (Airoldi & Beck, 2007; Thurstan et al., 2023; zu Ermgassen et al., 2025). Once widespread along the European coast, they have declined dramatically due to a combination of overfishing, habitat destruction, disease, and anthropogenic pressure (Airoldi et al., 2008; Pogoda, 2019; Thurstan et al., 2023). Today, they have almost entirely disappeared from their historical habitats, making them a priority for conservation and restoration. The species and the habitat are listed as threatened and/or declining by the Oslo-Paris-Commission (OSPAR) under the Convention for the Protection and Conservation of the North-East Atlantic (OSPAR, 2009), biogenic reefs are protected under EU Flora-Fauna-Habitat Directive (FFH Directive, 92/43/EWG) (Council of the European Union, 1992) and the Marine Strategy Framework Directive (MSFD) (Council of the European Union, 2008) and the habitat type reef (EU code 1170) is to be restored under the EU Nature Restoration Regulation (NRR) (OSPAR, 2009).

Restoring these habitats is not only an ecological necessity but also a unique opportunity to restore vital ecosystem functions (Waltham et al., 2020). *O. edulis* is a filter-feeder bivalve that plays a key role in maintaining water quality, promoting nutrient cycling, and increasing local biodiversity (zu Ermgassen et al., 2020; zu Ermgassen et al., 2016a). By forming biogenic reefs, these oysters provide shelter and substrate for a variety of marine species, acting as ecosystem engineers (OSPAR, 2009). Their presence creates structurally complex environments that can stabilize sediments and buffer coastal erosion (Preston et al., 2020; Thomas et al., 2022a). Consequently, restoring oyster populations contributes to both biodiversity enhancement (Benoit et al., n.d.; Preston et al., 2020; Zu Ermgassen et al., 2020) and climate change resilience (Lee et al., 2020; Thomas et al., 2022b).

Despite growing interest in restoration efforts, understanding of *O. edulis* biology, particularly in their natural environment, remains limited (Pogoda et al., 2020, 2023). Gaining insight into their phenology is crucial for identifying factors to strengthen and optimize restoration strategies. However, such knowledge has historically been difficult to obtain, as the marine environment presents numerous technical and logistical challenges for long-term, *in situ* monitoring. Existing research has primarily focused on aquaculture settings (Maneiro et al., 2017) and the reproductive biology of the species (Korringa, 1940; Orton, 1927). *O. edulis* is a gregarious reef-forming bivalve with a planktonic larval stage followed by permanent settlement on shell substrates preferably

conspecific shells, leading to the formation of complex biogenic reef structures (Bayne, 1969; zu Ermgassen et al., 2016b). Its valve activity, as in other filter-feeding bivalves, regulates feeding, respiration, and reproductive functions and is itself influenced by environmental conditions such as temperature, salinity, and suspended particulate matter (Gosling, 2003; Yonge, 1926). Recent studies have now investigated the *in situ* daily valve activity of *O. edulis* in both field (Tonk et al., 2023) and laboratory conditions (Le Moal, Tran, et al., 2025). These studies showed that valve behavior in *O. edulis* responds to environmental conditions and reflects key physiological processes such as feeding and respiration, but they remained limited to short-term or daily-scale investigations, leaving seasonal and long-term phenological rhythms largely unexplored. Thus, a significant knowledge gap still exists regarding how oysters behave and function in their natural, dynamic marine habitats.

Historically, oyster reefs were widespread along European coastal waters, from the Atlantic coast to the Mediterranean Sea, including the Black Sea (Thurstan et al., 2023). One of the most notable examples of this historical abundance was found in the North Sea, covering approximately 20,000 to 25,000 km^2 (Berghahn & Ruth, 2005; Korringa, 1940), particularly in the German Bight and around the island of Helgoland (Möbius, 1877). According to an estimate from the Biological Institute of Helgoland (BAH), the Helgoland oyster bed comprised about 1.5 million individuals in 1900, but as in many other regions, the species vanished entirely in the 1940s, due to overfishing, habitat destruction, and diseases, with no remaining natural populations (Beck et al., 2011; Gercken & Schmidt, 2014; Pogoda, 2019). Today, reintroduction and restoration of *O. edulis* in the German Bight contribute to the overarching objectives of ecosystem health, reversal of biodiversity loss and climate resilience (Küfeoğlu, 2022). In response, several initiatives have recently emerged at the European level, such as the Native Oyster Restoration Alliance (NORA), which has created a network of scientists, experts, and professionals dedicated to restoring *O. edulis* populations across Europe (Pogoda et al., 2019). In Germany, a restoration program has been underway since 2016 to develop and implement sustainable measures to restore European oyster reef habitats in Marine Protected Areas of the German Bight.

To ensure the long-term success of these efforts, it is important to investigate how *O. edulis* interacts with its environment on both biological and temporal levels. Like many organisms, oysters exhibit biological rhythms, that are governed by endogenous molecular clocks (Bell-Pedersen et al., 2005; Cermakian & Sassone-Corsi, 2000a; Dunlap, 1999; Partch et al., 2014a). These clocks are synchronized with external environmental cues and control a wide range of physiological and behavioral processes, such as feeding, metabolism, and reproduction. In marine environments, organisms face a complex set of

cyclical influences due to the overlapping effects of several astronomical cycles (Andreatta & Tessmar-Raible, 2020), including daily, tidal, lunar, semilunar, and annual cycles. The temporal coordination of biological processes with these cycles is crucial for an organism's health and fitness (Häfker et al., 2023; Helm et al., 2017; Mermet et al., 2017). In our investigations, we used valve behavior, shell growth, and biological rhythms at gene and behavioral scales as proxies for biological processes and their synchronization with environmental factors to estimate the oysters' fitness (Payton et al., 2017; Tran et al., 2011; Tran, Andrade, et al., 2020). Understanding how *O. edulis* responds to this complex temporal landscape is critical for identifying optimal conditions for growth, reproduction, and survival in natural settings.

This study aims to fill the knowledge gap regarding the phenology of *O. edulis* in its natural environment by investigating its annual valve behavior and shell growth, over an 18-months *in situ* monitoring period in the German Bight, using high-resolution valvometry under field conditions. This non-invasive technique uses electrodes attached to each shell to continuously record changes in inter-valve distance associated with valve opening amplitude. We monitored the oyster's valve activity *in situ* alongside environmental parameters including photoperiod, temperature, water level, underwater sound, chlorophyll *a* concentration, salinity, and turbidity. Additionally, we analysed the monthly gene expression in the gills and labial palps of 13 orthologous genes believed to be involved in the clock machinery, including 9 circadian clock genes (*OeClock*, *OeBmal1*, *OePer1*, *OeTim1*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeShag*, *OeCwo*), a clock-controlled metabolic gene (*OeNampt*), a gene involved in melatonin synthesis (*OeHiomt*), and photoreceptor genes (*OeCry1*, *OeOpn4*). These genes were selected based on their conserved roles in circadian clock function, photoreception, and temporal regulation previously described in vertebrates, insects, and marine invertebrates, including bivalves (Cermakian & Sassone-Corsi, 2000b; Le Moal, Payton, Tran, et al., 2025; Partch et al., 2014b; Perrigault & Tran, 2017).

We therefore sought to determine whether *O. edulis* exhibits synchronized behavioral, physiological, and molecular rhythms in its natural environment. We hypothesized that the expression of these rhythms across different temporal scales reflects the species' good health and could provide a valuable indicator for assessing the suitability of *O. edulis* restoration sites, such as Helgoland. This comprehensive chronobiological approach enables us to assess how behavioral and molecular rhythms align with environmental cycles and how they may be altered in a restored population. This work provides essential knowledge for a deeper understanding of *O. edulis* ecology and supports the development of evidence-based restoration strategies in complex dynamic marine ecosystems.

Material and methods

Experimental model

All research detailed in this study complied with German law, and the experiment was conducted according to international ethical standards. The experiment was realized in the underwater experimental field "Margate" (54° 11′ 35″ N, 7° 52′ 45″ E) off the island of Helgoland, Germany over a period of 540 days, from 11th March 2023 to 31st August 2024, involving 156 European native oysters *O. edulis* (approximately 8-10 years old, according to hatchery records). European native oysters were hatchery-reared individuals originating from broodstock collected at Loch Ryan in South West Scotland (54° 59′ 10″ N, 5° 03′ 18″ W) (Rossmore Oysters Ltd.), a sustainably managed source that represents an important broodstock for the Helgoland Oyster Hatchery and German restoration programs(Eagling et al., 2015). This broodstock was selected in accordance with current recommendations for sustainable oyster restoration and to preserve the genetic diversity of Northeast Atlantic populations used in restoration programs(Pogoda et al., 2019). Prior to field deployment, experimental work was conducted at the Biological Institute Helgoland of the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research (AWI). Upon arrival, all oysters were accompanied by official veterinary health certificates and had tested negative for *Bonamia* sp. and *Marteilia* sp. Prior to entering the hatchery system, oysters underwent the standard biosecurity protocol, including mechanical cleaning to remove sediment and epibionts, chemical disinfection (freshwater rinse, 0.2% sodium hypochlorite treatment, and final rinse with filtered UV-treated seawater). Following this biosecurity procedure, oysters were acclimated for 3 weeks in outdoor tanks under natural light conditions in a continuous flow-through system using natural seawater from the North Sea, supplemented with a laboratory-cultured microalgae mixture of *Rhodomonas salina, Isochrysis galbana* and *Chaetoceros muelleri*. After acclimation, oysters were transferred to the field (Figure 5.1). All underwater installations, maintenance checks and sampling of the oyster baskets were conducted by scientific divers from the AWI Centre for Scientific Diving (CSD).
The regulatory framework for research involving living marine organisms is provided by the Nature Reserve Ordinance *Helgoländer Felssockel* and the management plan for the Fauna-Flora-Habitat area DE-1813-391 Helgoland with *Helgoländer Felssockel*.
The study was divided into a behavioral and a molecular part.

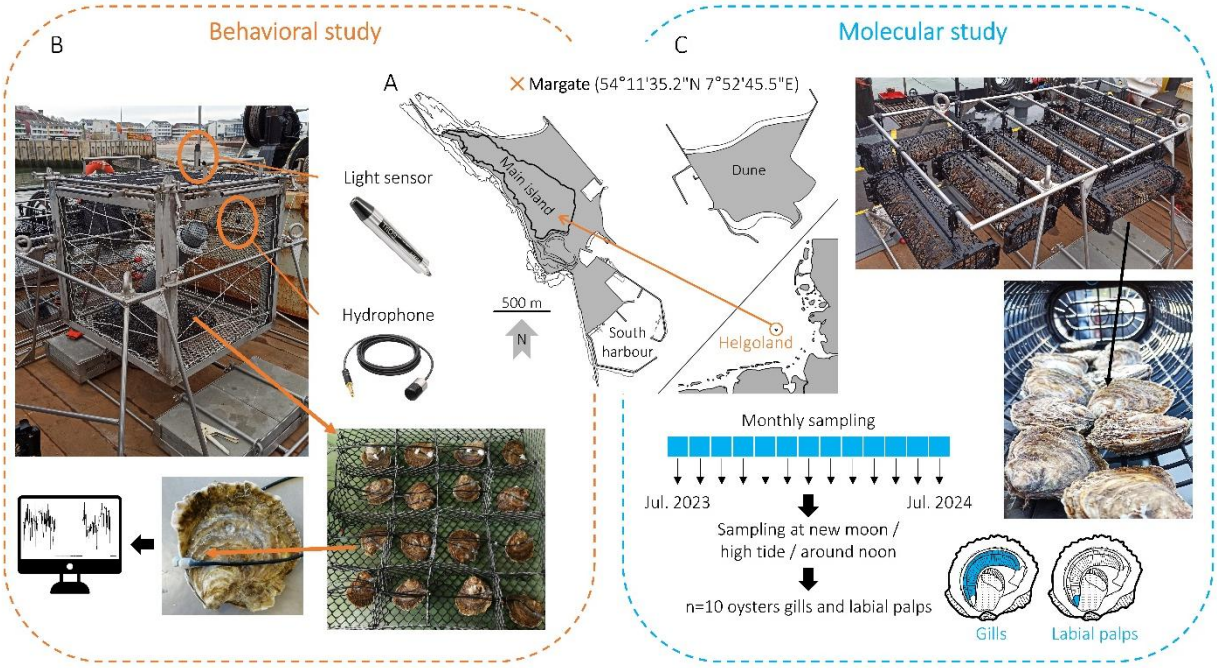


Figure 5.1. Experimental setup and protocol on Helgoland. (A) Study area (island of Helgoland) and its location in the German Bight, North Sea (bottom right corner). The orange cross represents the exact location of the experimental setup at the Margate (Teschke et al., 2020) (B) Experimental setup to investigate the behavioral rhythm of *O. edulis* from March 2023 to August 2024. Picture of the "valvometer lander", a metallic structure containing a light sensor, a hydrophone, an HFNI valvometer, a basket with 16 European native oysters equipped with electrodes to monitor their valve behavior continuously. (C) Experimental setup and protocol of the monthly sampling to investigate the molecular rhythm of *O. edulis* from July 2023 to August 2024. Pictures of the baskets containing oysters for the monthly sampling of oyster gills and labial palps, colored in blue in the diagrams (oyster diagrams: ©2020, Native Oyster Network - UK & Ireland, Native Oyster Restoration Alliance).

Behavioral part

Experimental setup

The behavioral part was conducted on 16 European flat oysters *O. edulis* with a mean ± standard deviation (SD) of 76.9 ± 4.6 mm shell length (range 69-84 mm) and 79.1 ± 4.9 mm shell width (range 69-86 mm) (Biometric measurements of all monitored individuals are provided in supplementary table S9.3). The oysters were divided into 16 individual chambers, which were positioned in a basket (Figure 5.1B). Each oyster was equipped with a pair of lightweight electrodes connected to a high-frequency non-

invasive (HFNI) valvometer (see below). The oyster basket was attached to a lander, which was deployed on 9th March 2023 at "Margate", at a depth of approximately 10m, with the help of the AWI Centre for Scientific Diving (CSD) (Figure 5.1A). The stainless-steel lander had a cubic structure with a volume of 838 cm^3. It was equipped with mesh frames on each side to protect the oysters from predators, without obstructing water flow (Fig. 5.1B). The mesh frames were replaced and cleaned monthly in parallel with the monthly samplings described below. The metallic structures showed no visible signs of corrosion or degradation throughout the experimental period, suggesting negligible galvanic corrosion effects on the oysters. Several sensors were integrated into the lander structure to measure environmental parameters simultaneously. Water temperature was measured every 10 seconds (ADT7420 sensor, AnalogDevices) near the valvometer. Underwater sound intensity was recorded in decibels SPL (dB re 1 µPa) using a hydrophone (Aquarian Audio Products (US), H2a) integrated into the HFNI valvometer system. Underwater sound was measured every 10 seconds across 16 selected low frequencies (10Hz, 20Hz, 30Hz, 40Hz, 50Hz, 60Hz, 70Hz, 80Hz, 90Hz, 100Hz, 200Hz, 300Hz, 400Hz, 500Hz, 600Hz et 700Hz) that are relevant to bivalve auditory sensitivity (Charifi et al., 2017). Light irradiance (W/m^2) was measured every 10 minutes in the 320nm - 950nm range with a radiometer (TRIOS ACC, RAMSES G2-ACC-VIS-300m), attached to the lander and pointing to the top (Fig. 5.1B). The resulting irradiance time series was used to identify light-dark transitions and estimate the daily photoperiod, defined here as the duration of the light phase (h/day). The radiometer also recorded water pressure at the sensor level every 10 minutes, providing continuous data in bars. These pressure values were then converted to water level using the conversion 1 bar = 10 meters of depth. In this study, we refer to water level rather than pressure, as the measured variation corresponds primarily to tidal cycles and secondarily to wave swell. Data were transferred daily to the AWI Centre for Scientific Diving (AWI CSD) using the "COSYNA underwater Node unit" connected via a sea cable to the land that provides power and internet connection to the valvometer and the sensors (Fischer et al., 2020). Hourly average values of temperature and underwater noise levels for each frequencies recorded throughout the experiment are publicly available in PANGAEA® - Data Publisher for Earth & Environmental Science (Le Moal, Payton, Geerebaert, et al., 2025). In parallel to our experiments, chlorophyll *a*, salinity and turbidity were recorded by the AWI-COSYNA Helgoland Underwater Observatory at "Margate" (Helgoland-UWO - 54°11.700'N / 7°52.600'E), which is equipped with a standard sensors unit providing real-time measures of environmental parameters (Baschek et al., 2017; Fischer et al., 2024, 2025). The environmental parameters monitored in this study were selected because they are known or suspected to influence biological rhythms and

physiological processes in bivalves. These include major environmental time cues such as photoperiod (Baumgartner & Tarrant, 2017; Helm et al., 2017; Ikegami & Yoshimura, 2017), temperature (Le Moullac et al., 2007; Young, 1979), and tidal cycles(Tran et al., 2011), as well as factors affecting feeding activity and physiological performance, including chlorophyll a concentration (used as a proxy for phytoplankton density) (Marescaux et al., 2016), underwater sound (Charifi et al., 2017; Ledoux et al., 2023), salinity (Pourmozaffar et al., 2020; Shumway, 1977), and turbidity (Tuttle-Raycraft & Ackerman, 2019).

Recording of valve behavior activity

The valve activity of 16 oysters was measured using a custom-built high-frequency non-invasive (HFNI) valvometer, allowing for the remote and continuous recording of their valve activity (Tran et al., 2023a). It consists of a pair of lightweight (custom-made) electrodes (<100 mg) designed to minimize disturbance of the oyster's behavior. The electrodes are glued to each half-shell, and each electrode is linked to the HFNI valvometer by a flexible wire allowing undisturbed oyster valve movement (Fig. 5.1B). An electromagnetic field is generated successively by each emitting electrode, and the signal received by the corresponding receiving electrode varies with the inter-valve distance, allowing the measurement of each oyster's valve activity every 1.6 seconds. Further details are given in Tran et al., 2023b and Le Moal et al., 2023. The daily recordings were processed using Labview 8.0 (National Instrument, Austin, TX, USA).
To analyze the behavioral rhythm, two parameters of valve activity were measured: the valve opening duration (VOD) and the valve opening amplitude (VOA). For each hour, the individual VOD and VOA are measured. VOD is established from an opening threshold (5% of VOA), setting the state of the animal as "open" or "closed". If the oyster is continuously open for an hour, the percentage of hourly VOD is 100%. On the contrary, if it does not open at all for an hour, the hourly VOD is 0%. Individual VOA of each oyster is reported each hour as a percentage, where 100 % indicates that the valves opened at their maximum amplitude during the whole hour and 0 % indicates that the valves were closed over the entire hour; in between all the values of amplitudes are possible. Maximum valve opening was defined empirically as the highest opening amplitude recorded for each individual during the monitoring period, with the reference value updated when new maxima were observed. All individual hourly valve behavior data (VOA and VOD) recorded throughout the experiment in Helgoland are publicly available in PANGAEA® - Data Publisher for Earth & Environmental Science (Le Moal, Payton, Geerebaert, et al., 2025).

The mean hourly VOA and VOD are represented as double-plotted actograms and heatmaps generated in Microsoft Excel using conditional formatting, where each line represents two days. In heatmaps, the mean hourly VOA and VOD are represented using a color scale from 0% to 100%, divided into eleven intermediate ranges. A specific category was assigned to 0%, corresponding to complete valve closure (purple), while values >0% were divided into ten equal ranges. This discretization was chosen to better distinguish fully closed states from minimal valve opening activity. In an actogram, hourly activity levels superior to the daily median are represented by black bars. In contrast, hourly activity levels inferior to the daily median are represented by light grey bars.

Measuring of daily shell growth

Individual daily shell growths were measured using the HFNI valvometer. Shell growth in bivalves is driven by the mantle, which deposits successive layers of calcium carbonate on the inner surface of the shell. Therefore, to measure individual daily shell growth, we recorded the minimal distance between electrodes placed on each oyster's valves when fully closed, over the 540 days of the experiment. The relative slopes of shell growth were calculated as a percentage for the studied periods. Zero per cent and 100% of growth corresponded to the minimal distance in mm between the electrodes at the beginning and the end of the experiment, respectively. Absolute shell growth, in millimetres (mm) was then estimated by measuring the individual shell lengths at the start and end of the experiment. For each oyster, the relative scale (0–100%) was linearly converted to actual shell growth (in mm), where 0% corresponded to the initial shell length and 100% to the final shell length, thus enabling the translation of the distance between electrodes when valves are closed into daily absolute shell length growth.

Molecular part

For the molecular part of this field experiment, 156 oysters were placed in 13 oyster baskets, with 12 oysters per basket. The 15 L baskets (SEAPA) had dimensions of $600 \times 140 \times 260$ mm and a mesh size of 6 mm. They were attached to a stainless-steel lander, which was deployed at "Margate", north of Helgoland, on 9th March 2023 (Figure 5.1A). From July 2023 to July 2024, oysters were sampled monthly, with a target sample size of 10 individuals per sampling event. Due to natural mortality, the number of oysters collected varied among sampling dates and ranged from 8 to 12 individuals. All sampled oysters were measured prior to molecular analyses and had a mean shell length of 85.6 ± 8.0 mm SD (range 70-110 mm) and a mean shell width of 80.1 ± 9.2 mm SD (range 42-105 mm) (Biometric measurements of all sampled individuals are provided in

supplementary table S9.4). All sampling was conducted around the 3rd quarter moon (± 3 days), low tide (± 3 hours), and around solar noon (± 2 hours) to minimise the varying influence of lunar, tidal, and daily rhythms on gene expression. Due to weather conditions, sampling was possible at each time point, except for January and February 2024, during which sampling occurred during the 1st quarter moon, low tide, and around solar noon.

Underwater, for each sampling event, one oyster basket was detached from the lander and quickly transferred to the boat where the sampled oysters were placed on dry ice. Oysters were euthanized by rapid freezing on dry ice immediately after sampling to minimize transcriptional changes associated with handling stress prior to tissue preservation. Then, back in the lab, each oyster was numbered, measured, and gills and labial palps were immediately dissected (Fig. 5.1C). Gills and labial palps were selected because they are directly involved in feeding and respiration and have been previously used in bivalves to investigate light-and rhythm-related molecular processes (Perrigault & Tran, 2017; Tran, Perrigault, et al., 2020a; Zhu et al., 2022). For gills, a tissue sample (0.12g) was taken and immediately immersed in 800 µl of RNA Later (Invitrogen™ RNAlater™ Stabilization Solution, Fisher Scientific). For labial palps, the four palps were transferred into 1000 µl of RNA Later (Invitrogen™ RNAlater™ Stabilization Solution, Fisher Scientific). Samples were then stored at 4°C for 24 hours before being transferred to -80°C for long-term preservation before laboratory analysis.

RNA extraction and cDNA synthesis

Total RNA was extracted from gills using Trizol (Invitrogen) and a Direct-zol™ RNA MiniPrep (Zymo Research). The quantity and quality of total RNA were assessed by spectrophotometry (OD230, OD260, OD280). RNA reverse transcription was performed using RevertAid H Minus First Strand cDNA Synthesis Kits (Thermo Scientific).

mRNA expression analysis by Real-Time PCR

Primers for clock and clock-associated genes (*OeClock*, *OeBmal1*, *OePer1*, *OeTim1*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeShag*, *OeCwo*, *OeNampt*, *OeHiomt*, *OeCry1*, *OeOpn4*) as well as housekeeping genes (*OeGapdh*, *OeEf1*, *Oe28S*) were designed using the coding sequences (CDS) of these genes, with the assistance of Primer3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). *OeCry2* was not included in this study because it was identified and characterized in *O. edulis* only after the primer panel and molecular analyses had been designed (Le Moal, Payton, Tran, et al., 2025). Forward and reverse sequences for the primers are detailed in supplementary table S9.5. Real-time quantitative PCR (qPCR) was performed with the PowerUp™

SYBR™ Green Master Mix kit (Fisher Scientific). The qPCR reactions were conducted under the following conditions: an initial activation step at 95°C for 2 minutes to activate the Dual-Lock™ DNA polymerase, followed by 40 cycles of denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute to amplify the target cDNA. After amplification, melting curves were generated by gradually decreasing the temperature from 95°C to 60°C to verify primer specificity. Relative transcript levels of clock and clock-associated genes were determined using the comparative Ct method ($2^{-\Delta Ct}$) as described by Livak and Schmittgen (Livak & Schmittgen, 2001), where ΔCt is calculated as $Ct_{(target\ gene)} - Ct_{(housekeeping\ gene)}$. Following the recommendations of Vandesompele et al., 2002 and the MIQE guidelines (Bustin et al., 2009), the stability of the reference genes was assessed using the geNorm algorithm, and gene expression was normalized using the geometric mean of the housekeeping genes *OeEf1*, *OeGapdh* and *Oe28S*.

Data analysis and Statistics

Mixed models

The influence of eight environmental parameters: photoperiod (daylength), photoperiod direction (increasing or decreasing photoperiod duration), temperature, water level, underwater sound, chlorophyll *a* concentration, salinity, and turbidity, on valve activity (VOA and VOD, individual hourly datasets) and shell growth (individual daily datasets) was investigated using linear mixed-effects models (LMMs). All models included individual identity as a random effect to account for intra-individual variability.

A two-step modelling approach, similar to the method described by Jamil et al., 2012, was applied. This strategy is widely employed in environmental science to investigate complex relationships between species and their environments. In the first step, we fitted global models for each response variable without interaction, including all eight environmental variables as fixed effects:

VOA or VOD or Growth ~ Photoperiod + Photoperiod direction + Temperature + Water level + Sound + Chlorophyll a + Salinity + Turbidity + (1 | Individual)

Underwater sound data were unavailable after 28 February 2024 due to a technical failure; therefore, this variable was not available for the corresponding period and was handled accordingly within the models.

This model (the Single Trait-Environment Relationships model) allowed us to assess the individual effects of each predictor and identify the most influential environmental variables. Prior to model fitting, collinearity among explanatory variables was assessed using pairwise Pearson correlation coefficients. Correlations among predictors were low

to moderate ($|r| < 0.5$), indicating no problematic collinearity among the environmental variables. Then, the three most significant predictors (based on χ^2 values) were retained for the second modelling step. In the second step, these three variables were used to construct a more focused model, including all two-way interactions:
VOA or VOD or Growth ~ Var1 + Var2 + Var3 + Var1:Var2 + Var1:Var3 + Var2:Var3 + (1 | Individual)
This Multiple Trait-Environment Relationships model allowed us to explore interactions between key environmental factors while reducing model complexity and limiting potential multicollinearity issues associated with the inclusion of the eight predictors and their interactions (Jamil et al., 2012, 2013).
All analyses were conducted using R (version 4.2.2) (R Core Team, 2023) using the packages "lme4" (Bates et al., 2015) and "car" (Fox & Weisberg, 2018) to get the significance of the test. A significance p-value of 0.05 was used for all analyses.

Chronobiological analysis

For behavioral data, chronobiological analyses were performed using the software Time Series Analysis Serial Cosinor 6.3. Several steps were necessary to validate a significant rhythm. First, to ensure data quality, the absence of random distribution in the dataset is checked using an autocorrelation diagram, and the absence of a stationary phenomenon is assessed through a partial autocorrelation function calculation (Box et al., 1994). Next, the data were examined for periodicity through spectral analyses, using the Lomb and Scargle periodogram (Scargle, 1982). This periodogram establishes a significance threshold ($p = 0.95$). Finally, rhythmicity is confirmed by the Cosinor model, utilizing the period identified by the Lomb and Scargle periodogram (Bingham et al., 1982). For a given period, the model is expressed as: $Y(t) = A\cos(\pi t/\tau + \varphi) + M + \varepsilon(t)$, where A represents the amplitude, φ is the acrophase, τ denotes the period, M is the mesor, and ε is the relative error. Two critical tests verify the validity of the calculated model and the existence of a rhythm: the elliptic test must be rejected, and the probability for the null amplitude hypothesis must be < 0.05. Rhythmicity in the circalunar range ($\tau = 29.5 \pm 3$ d) and the circasemilunar range ($\tau = 14.8 \pm 1.5$ d) were investigated over the entire 540-day experiment. In contrast, circadian ($\tau = 24 \pm 4$ h) and circatidal ($\tau = 12.4 \pm 2$ h) rhythms were evaluated over full seasons with 15 individuals: spring (20/03/2023 to 20/06/2023), summer (21/06/2023 to 22/09/2023), autumn (23/09/2023 to 21/12/2023) and winter (22/12/2023 to 19/03/2024). These analyses were conducted for both valve opening amplitude (VOA) and valve opening duration (VOD) at the individual level (n = 15 individuals).

Rhythmic analysis of circannual rhythm in valve activity (VOA and VOD) and clock genes expression was carried out using the R package 'RAIN' (R Core Team, 2023; Thaben & Westermark, 2014). RAIN was specifically designed to detect rhythms in biological datasets, regardless of waveform, employing a non-parametric approach (Thaben & Westermark, 2014). Analyses were performed on monthly mean values of valve activity and on gene expression data obtained from the monthly sampling. For both datasets, the presence of a significant annual rhythm was tested by specifying a period of 12 months. Rhythmicity was considered significant for a corrected p-value less than or equal to 0.05.

Temporal segmentation for biological rhythm analysis

To assess variations in valve activity (hourly VOA and VOD) across different biological rhythms, the dataset was segmented according to specific geophysical cycles studied during the chronobiological analysis. For the analysis of annual rhythms, data were divided by season: spring (11/03/2023 to 20/06/2023 and 20/03/2024 to 19/06/2024), summer (21/06/2023 to 22/09/2023 and 20/06/2024 to 31/08/2024), autumn (23/09/2023 to 21/12/2023), and winter (22/12/2023 to 19/03/2024). For lunar rhythms, VOA and VOD values were averaged over a 3-day window centered on each main lunar phase (new moon, first quarter, full moon, and third quarter), with lunar dates retrieved from the website www.timeanddate.com. For the semilunar rhythm (neap-spring tidal cycle), data were averaged over 3-day periods centered on key tidal coefficient phases (high, decreasing, low, and increasing coefficients), with tidal coefficients from Helgoland obtained from the website https://mareespeche.com. For daily rhythms, VOA and VOD were averaged separately for daytime and nighttime periods, defined according to theoretical sunrise and sunset times for each season used in the chronobiological analysis (retrieved from the website www.timeanddate.com). Lastly, for tidal rhythms, for each season used in the chronobiological analysis, data were categorized into four 6-hour phases based on *in situ* pressure recordings: high tide (6 h of the highest water level), low tide (6 h of the lowest water level), ebb (decreasing water level), and flow (icreasing water level). This segmentation allowed for targeted analysis of valve activity aligned with relevant chronobiological cycles.

Descriptive statistics

For each temporal segmentation, including annual, lunar, semilunar, daily, and tidal rhythms, differences in VOA and VOD values across the designated phases were tested using the non-parametric Kruskal-Wallis test. This test was chosen because it does not assume normality and is suitable for comparing multiple independent groups with

potentially non-Gaussian distributions. When the Kruskal-Wallis test revealed significant differences, pairwise comparisons were conducted using Dunn's post hoc test, which accounts for multiple comparisons while maintaining robustness for non-parametric data. All analyses were conducted using R (version 4.2.2) (R Core Team, 2023) and a significance p-value of 0.05 was applied for all analyses.

Results

Ostrea edulis valve activity

We continuously monitored the valve activity of *Ostrea edulis* over 540 days in the field. Figure 5.2 shows the temporal dynamics of valve activity, presented as double-plotted heatmaps and actograms for both VOA (left panel) and VOD (right panel). Hourly mean values are overlaid with photoperiod transparency to aid in temporal interpretation. The corresponding daily valve activity of each individual oyster is presented in Supplementary Figure S9.9. One oyster died one day after deployment; the remaining 15 oysters survived the entire experiment (540 days). However, due to technical issues, valve activity was recorded for 15 oysters until March 2024, then for 14 oysters until June 2024, and finally for 8 oysters until the end of the experiment. Despite this progressive reduction in sample size due to technical signal loss, the high-frequency continuous recordings yielded numerous repeated measurements per individual, helping maintain robust estimates of valve activity dynamics throughout the monitoring period.

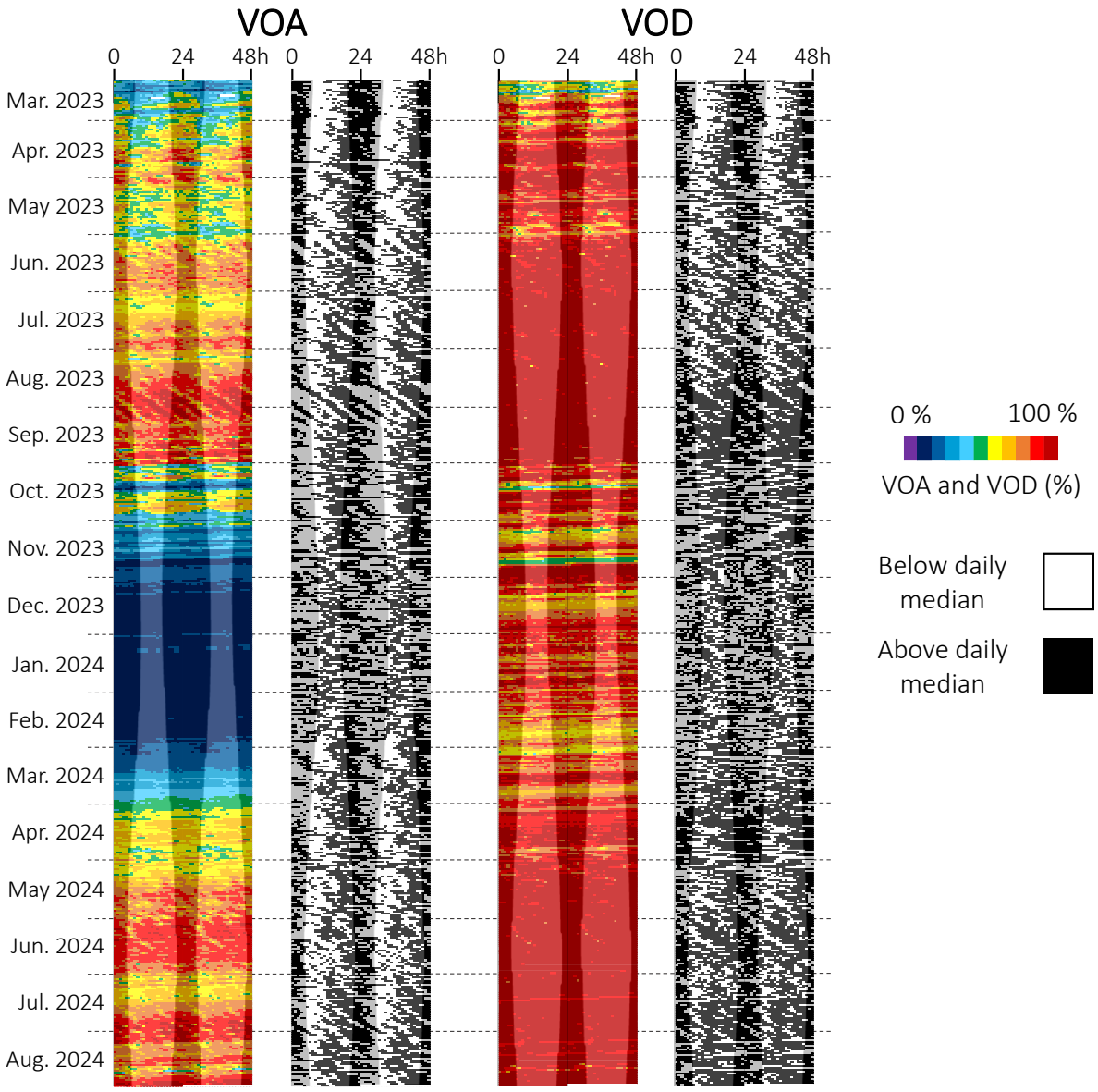


Figure 5.2. *Ostrea edulis* hourly valve activity in the field over the 540 experimental days. Double-plotted heatmaps and associated actograms of mean valve opening amplitude (VOA, %, left panel) and valve opening duration (VOD, %, right panel) from 11th March 2023 to 31st August 2024 in the island of Helgoland, Germany (n = 8-15; 15 oysters until March 2024, 14 until June 2024, and 8 thereafter due to progressive technical issues). Photoperiod is represented in the heatmaps and actograms in transparent shading. In heatmaps, hourly VOA and VOD are shown using a 0-100% color scale, with 0% (complete closure) displayed as a distinct category (purple) and values >0% grouped into 10 equal intervals.

The heatmaps reveal a clear annual pattern of VOA, with values gradually increasing from early spring (March 2023) to peak in summer (*e.g.*, with mean monthly VOA values reaching 77 ± 2% in August and 81 ± 1% in September 2023), then declining steadily from the start of autumn to late autumn (November 2024), and finally reaching a minimum in winter (as low as 7 ± 1% of monthly VOA in January). After winter 2024, the valve opening increases again until summer, gradually rising from VOA from 40 ± 2% in March to 83 ± 1% in June, following trends similar to those seen in spring and summer 2023 with peak values reached around late July / early August 2024. VOD showed a much less pronounced seasonal pattern. VOD remains nearly maximal throughout the year (88 ± 14%), except during autumn and winter, when it drops to 40 ± 3% and to 53 ± 1% of mean daily VOD, respectively. The VOA and VOD actograms reveal underlying rhythmic patterns in valve activity at the daily and tidal scales that vary seasonally. Clear patterns are visible from March 2023 to mid-November 2023, and again from early March 2024 to the end of the experiment, while a more irregular blurred phase is observed in between, corresponding to the winter period. During periods of pronounced rhythmicity, a consistent diel pattern is evident, often accompanied by an underlying tidal signal that occasionally becomes more prominent, particularly noticeable in early June and late August 2023 (see representative examples in Supplementary Figure S9.10).

Environmental parameters

To understand the environmental factors affecting the biological rhythms of *Ostrea edulis* in the field, we monitored seven abiotic parameters over the 540-day experiment: temperature, photoperiod, water level, underwater sound, chlorophyll *a* concentration, salinity, and turbidity. Collectively, these environmental measurements offer a comprehensive framework for assessing the natural entrainment of biological rhythms and physiological processes in *O. edulis* under natural conditions.

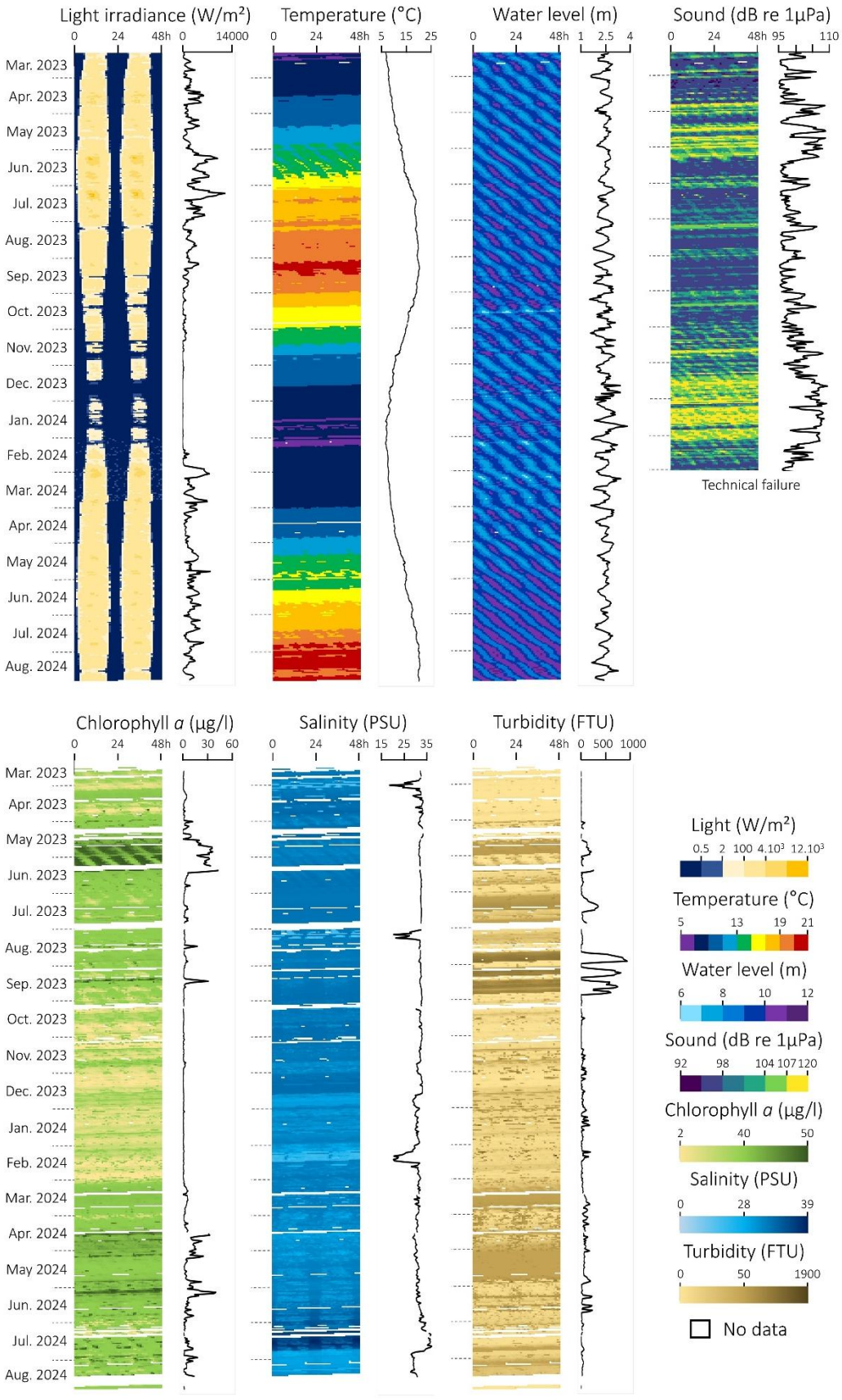

Light irradiance (W/m²)
Temperature (°C)
Water level (m)
Sound (dB re 1µPa)
Technical failure
Chlorophyll a (µg/l)
Salinity (PSU)
Turbidity (FTU)
Mar. 2023
Apr. 2023
May 2023
Jun. 2023
Jul. 2023
Aug. 2023
Sep. 2023
Oct. 2023
Nov. 2023
Dec. 2023
Jan. 2024
Feb. 2024
Mar. 2024
Apr. 2024
May 2024
Jun. 2024
Jul. 2024
Aug. 2024
Light (W/m²)
Temperature (°C)
Water level (m)
Sound (dB re 1µPa)
Chlorophyll a (µg/l)
Salinity (PSU)
Turbidity (FTU)
No data

Figure 5.3. Time series of environmental parameters monitored over the 540 experimental days. Time series of light irradiance (W/m²), temperature (°C), water level variation (m), underwater sound (dB re 1µPa), chlorophyll *a* concentration (µg/l), salinity (PSU) and turbidity (FTU) monitored from 11th of March 2023 to 31st of August 2024 in the island of Helgoland, Germany. For each parameter, a double-plotted heatmap of hourly means is shown on the left, and a line chart of daily means on the right. For the light, the line chart shows the daily maximum value. For the water level, the line chart illustrates the difference between the daily maximum and minimum values. The underwater sound dataset ends on 28/02/2024 due to a technical failure. White cells represent missing data.

Photoperiod followed the solar cycle, ranging from 7.5 hours of daylight at the winter solstice to 17.5 hours at the summer solstice, with maximum measured light irradiance reaching 11,979 W/m² at the oyster's level in July 2023. Temperature exhibited a clear seasonal pattern, gradually rising from winter to summer, reaching 20.7 °C in August 2024. The lowest temperature recorded was 6.42 °C in February 2024. Seasonal averages for 2023/2024 were 11.2 ± 0.1 °C in spring, 18.8 ± 0.1 °C in summer, 14.2 ± 0.1 °C in autumn, and 7.7 ± 0.1 °C in winter. The water level above the oysters fluctuated regularly between 6 and 12 meters, driven by tidal cycles and wave swell, with a daily mean oscillation of 2.5 ± 0.1 meters. Underwater sound levels exhibited episodic peaks, particularly between mid-May and mid-June 2023 and from December 2023 to January 2024, with intensities ranging from 107 to 120 dB re 1 µPa. Chlorophyll *a* concentration had notable spring peaks, with daily maxima of 43 µg/L on 13 June 2023 and 40 µg/L on 9 June 2024. Seasonal averages were highest in spring (9.5 ± 0.2 µg/L), and much lower in autumn (1.1 ± 0.1 µg/L) and winter (1.0 ± 0.1 µg/L). Salinity remained relatively constant at around 31.4 ± 0.1 PSU, with lower values during rainy winter periods and higher levels in summer (*e.g.* July 2024). Turbidity exhibited both seasonal and episodic variability, with notable increases in August-September 2023 and May 2024. Average seasonal values were highest in summer (140.1 ± 4.0 FTU) and spring (60.7 ± 1.4 FTU), and lowest in autumn (49.5 ± 2.9 FTU) and winter (42.2 ± 2.5 FTU) (Fig. 5.3).

The influence of environmental parameters on *Ostrea edulis* valve activity

Linear mixed-effects models were used to assess the influence of environmental factors on hourly VOA et VOD. The outputs of the Simple Trait-Environment Relationships and Multiple Trait-Environment Relationships models are summarized in Table 1. In the first VOA model, all parameters demonstrated significant effects. Photoperiod had

the most influential impact (χ^2 = 22010), followed by temperature (χ^2 = 11702) and sound (χ^2 = 6310). The effects of salinity (χ^2 = 948), chlorophyll *a* (χ^2 = 495), and turbidity (χ^2 = 437) were also significant, while water level effects were marginal (χ^2 = 21). Positive effects were observed for photoperiod (β = 4.10), temperature (β = 3.45), and water level (β = 3.26), indicating that an increase in these variables is associated with higher VOA; conversely, decreasing photoperiod (β = -12.43) and sound (β = -1.26) had negative effects. In the second VOA model, which focused on temperature, photoperiod, and sound, all three environmental factors were significant, both individually and in interaction. The interaction between temperature and photoperiod had the most decisive influence (χ^2 = 7788), followed by temperature (χ^2 = 6087), and the interaction between temperature and sound (χ^2 = 3366), highlighting a pronounced synergistic effect between temperature and photoperiod or sound, which overshadows the independent influence of photoperiod. For VOD, the first model without interaction revealed significant effects for all parameters except salinity (p = 0.081). Temperature (χ^2 = 802), photoperiod (χ^2 = 573), and chlorophyll *a* (χ^2 = 215) were the strongest predictors. Photoperiod direction (χ^2 = 186), turbidity (χ^2 = 144), sound (χ^2 = 104), and water level (χ^2 = 17) had lower, yet significant effects. The second VOD model, incorporating temperature, photoperiod, and chlorophyll *a*, indicated, that similar to VOA, the predominant effect was a synergic interaction between temperature and photoperiod (χ^2 = 717), followed by the individual effects of temperature (χ^2 = 338) and photoperiod (χ^2 = 157), as well as interactions between chlorophyll *a* and temperature (χ^2 = 27) and chlorophyll *a* and photoperiod (χ^2 = 28).

Table 5.1. Results from linear mixed models analyzing the effect of environmental parameters on *Ostrea edulis* valve activity (VOA and VOD). Outputs from linear mixed models considering the influence of various fixed factors (photoperiod, photoperiod direction, water temperature, water level, underwater sound, chlorophyll *a* concentration, salinity, and turbidity) on the individuals' VOA and VOD over the study period (540 days, i.e. 12960 hours). A second model examines the influence of the three most significant predictors from the first model (based on χ^2) without interaction, as well as all two-way interactions, on individuals' VOA and VOD over the study period.

VOA					**VOD**				
Single Trait-Environment Relationships model									
VOA ~ Photop. + Photop. dir. + Temp. + Water level + Sound + Chl. *a* + Salinity + Turbidity + (1 | Ind)					VOD ~ Photop. + Photop. dir. + Temp. + Water level + Sound + Chl. *a* + Salinity + Turbidity + (1 | Ind)				
Parameters	*β*	*SE*	*χ2*	*p*	Parameters	*β*	*SE*	*χ2*	*p*
Photop.	4.10	0.03	22010	<0.001	Photop.	1.01	0.04	573	<0.001
Photop. dir.	-12.43	0.26	2362	<0.001	Photop. dir.	-5.34	0.39	186	<0.001

Temp.	3.45	0.03	11702	<0.001	Temp.	1.38	0.05	802	<0.001
Water level	3.26	0.72	21	<0.001	Water level	-4.47	1.10	17	<0.001
Sound	-1.26	0.02	6310	<0.001	Sound	-0.25	0.02	104	<0.001
Chl. *a*	-0.18	0.01	495	<0.001	Chl. *a*	-0.18	0.01	215	<0.001
Salinity	0.66	0.02	948	<0.001	Salinity	-0.06	0.03	3	0.081
Turbidity	0.01	0.00	437	<0.001	Turbidity	0.01	0.00	144	<0.001
Multiple Trait-Environment Relationships model									
VOA ~ Photop. + Temp. + Sound + Temp.:Photop. + Temp.:Sound + Photop.:Sound + (1 | Ind)					VOD ~ Photop. + Temp. + Chl. *a* + Temp.: Photop. + Temp.:Chl. *a* + Photop.:Chl. *a* + (1 | Ind)				
Photop.	12.87	0.53	600	<0.001	Photop.	-1.42	0.11	157	<0.001
Temp.	31.89	0.41	6087	<0.001	Temp.	-1.97	0.11	338	<0.001
Sound	1.88	0.06	960	<0.001	Chl. *a*	0.27	0.20	2	0.185
Temp. x Photop.	-0.54	0.01	7788	<0.001	Temp. x Photop.	0.23	0.01	717	<0.001
Temp. x Sound	-0.23	0.00	3366	<0.001	Temp. x Chl. *a*	0.03	0.01	27	<0.001
Photop. x Sound	-0.01	0.01	5	0.021	Photop. x Chl. *a*	-0.05	0.01	28	<0.001

VOA: valve opening amplitude; VOD: valve opening duration; Photop.: photoperiod (daylength); Photop. dir.: photoperiod direction (increasing or decreasing daylength); Temp.: water temperature; Chl. *a*: chlorophyll *a* concentration.

Ostrea edulis shell growth

Shell growth was monitored daily throughout the 540-day experiment. Figure 5.4 displays the mean (± SE) cumulative shell growth curves. For visualization purposes, the curve is color-coded according to the mean daily VOA, which showed a stronger association with shell growth than VOD and therefore facilitated visualization of their seasonal relationship. The 2023 growing period peaked in summer, then declined in autumn and winter, with a renewed increase starting in spring 2024, particularly in the summer of 2024. The reduced growth observed during spring 2023 reflects interannual variability in the timing of the growth season rather than a lack of growth. While growth onset occurred later in 2023 than in 2024, both years exhibited a similar overall seasonal pattern and a comparable total shell increment. This shift is likely driven by interannual differences in environmental conditions (e.g. temperature, food availability, and light regime) rather than acclimatization, as oysters were already fully acclimated prior to deployment and no abnormal valve behavior or evidence of technical artefacts were detected during this period. Seasonal variations in VOA broadly paralleled the observed growth phases, with higher VOA values occurring during periods of steeper shell growth. Consistent with this pattern, a significant positive correlation was observed between VOA and shell growth (Spearman test: $p = 0.0129$). The increase in SE toward the end of the experiment reflects the reduced number of individuals following progressive technical loss of signals.

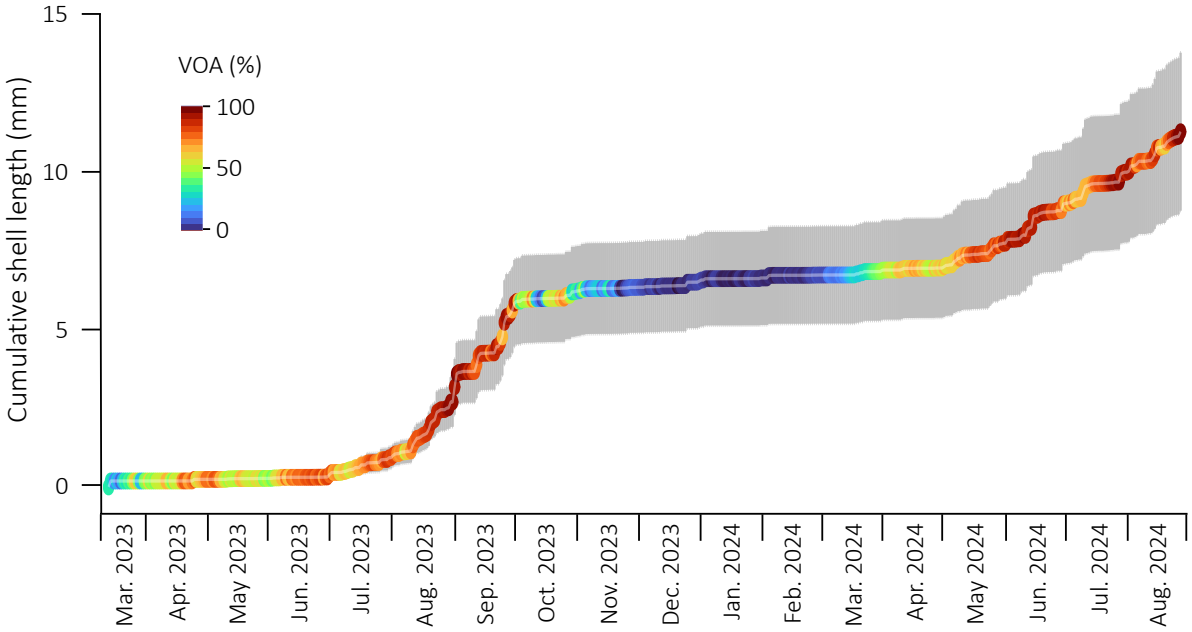


Figure 5.4. Daily shell growth of *Ostrea edulis* in the field over 540 days of experimentation. Line chart showing the cumulative shell growth of all individuals (n = 8-15; 15 oysters until March 2024, 14 until June 2024, and 8 thereafter due to progressive technical issues) from 11th March 2023 to 31st August 2024 on the island of Helgoland, Germany. The curve is colored by the daily mean valve opening amplitude (VOA, %) to facilitate visual comparison between seasonal patterns of valve activity and shell growth. Error bars represent the standard error (SE).

The influence of environmental parameters on *Ostrea edulis* shell growth

Linear mixed-effects models were used to assess the influence of environmental factors on shell growth. The outputs of the Simple Trait-Environment Relationships and Multiple Trait-Environment Relationships models are summarized in Table 2. In the first model, temperature ($\chi^2 = 63$) was the strongest predictor, followed by photoperiod direction ($\chi^2 = 16$) and photoperiod ($\chi^2 = 12$). Chlorophyll *a* ($\chi^2 = 7$) and water level ($\chi^2 = 4$) had minor significant effects, whereas sound, turbidity, and salinity were non-significant. The second model, which tested temperature, photoperiod, and photoperiod direction, as well as their interactions, showed that the interaction between temperature and photoperiod direction ($\chi^2 = 13$) and photoperiod direction alone ($\chi^2 = 10$) had the most potent effects on daily shell growth. The synergy between photoperiod and photoperiod direction ($\chi^2 = 5$) and the impact of photoperiod alone ($\chi^2 = 4$) are also significant, while the effects of temperature alone and in interaction with photoperiod are not significant.

Table 5.2. Results from linear mixed models examining the influence of environmental parameters on *Ostrea edulis* shell growth. Outputs from linear mixed models consider the influence of different fixed factors (photoperiod, photoperiod direction, water temperature, water level, underwater sound, chlorophyll *a* concentration, salinity, and turbidity) on the individuals' daily shell growth over the study period (540 days) for the first model. A second model, explores the influence of the three most significant predictors from the first model (based on χ^2) along with all two-way interactions on the individuals' shell growth during the study period.

Growth				
Single Trait-Environment Relationships model				
Growth ~ Photop. + Photop. dir. + Temp. + Water level + Sound + Chl. *a* + Salinity + Turbidity + (1 | Ind)				
Parameters	*β*	*SE*	*χ2*	*p*
Photop.	-0.01	0.00	12	<0.001
Photop. dir.	-0.04	0.01	16	<0.001
Temp.	0.01	0.00	63	<0.001
Water level	0.12	0.06	4	**0.047**
Sound	0.01	0.00	0	0.483
Chl. *a*	-0.01	0.00	7	**0.010**
Salinity	0.01	0.00	1	0.358
Turbidity	0	0.00	0	0.735
Multiple Trait-Environment Relationships model				
Growth ~ Photop. + Photop. dir. + Temp. + Temp.: Photop. + Temp.:Photop. dir. + Photop.:Photop. dir. + (1 | Ind)				
Photop.	-0.01	0.00	4	**0.05**
Photop. dir.	-0.07	0.02	10	**0.001**
Temp.	-0.01	0.01	2	0.13
Temp. x Photop.	0.00	0.00	3	0.07
Temp. x Photop. dir.	0.01	0.00	13	<0.001
Photop. x Photop. dir.	-0.01	0.00	5	0.02

Photop.: photoperiod (daylength); Photop. dir.: photoperiod direction (increasing or decreasing daylength); Temp.: water temperature; Chl. *a*: chlorophyll *a* concentration.

Biological rhythms of *Ostrea edulis* valve behavior

Chronobiological analysis revealed how *O. edulis* synchronizes its behavior with various geophysical cycles (Fig. 5.5). We assessed rhythmicity in VOA and VOD across annual, lunar, semilunar, daily, and tidal cycles using RAIN and Cosinor analyses (Fig. 5.5A, B).

For VOA (Fig. 5.5A, C), significant annual rhythms (12-month cycle) were observed in 80% of individuals (Fig. 5.5A), with the highest VOA in summer and the lowest in

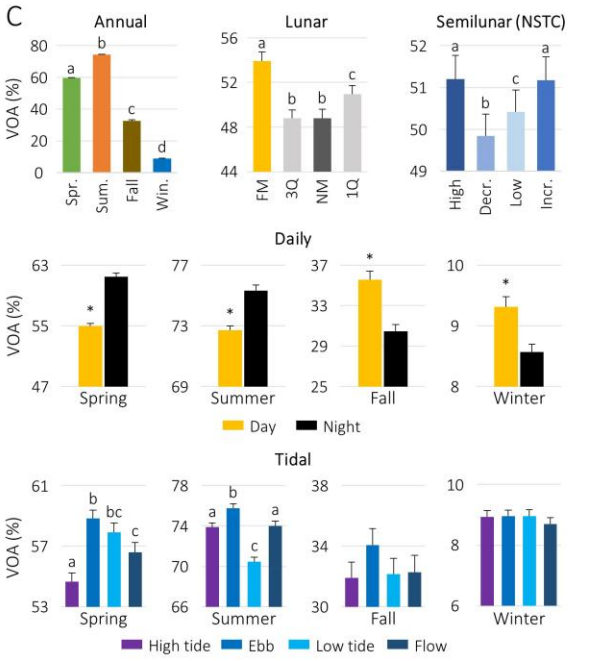
winter (Fig. 5.5C). Lunar rhythms of 29.2 ± 0.7 days, aligned with the ~29.5 days synodic cycle of the moon, were detected in 67% of individuals (Fig. 5.5A). Phase analysis showed that VOA peaked at full moon, was intermediate at first quarter moon, and lowest at new moon and third quarter moon (Fig. 5.5C). Semilunar rhythms appeared in 60% of individuals with an average period of 14.7 ± 0.6 days, matching the ~14.7 days semilunar cycle (Fig. 5.5A). VOA was highest during high and increasing tides (Fig. 5.5C). Daily rhythms, following a 24-hour cycle, were observed across all seasons. However, the proportion of individuals showing significant daily rhythms was high in spring and summer (93% in both) and markedly lower in autumn and winter (13% and 7%, respectively) (Fig. 5.5A). Additionally, individuals were significantly nocturnal in spring and summer, shifting to diurnal patterns in autumn and winter (Fig. 5.5C). Tidal rhythms, aligned with a 12.4-hour cycle, were evident in spring and summer (60% and 47% of rhythmic individuals, respectively), but absent in autumn and winter (Fig. 5.5A). During spring and summer, the highest activity levels coincided with ebb tides (Fig. 5.5C).

For VOD (Fig. 5.5B, D), similar patterns were observed. Annual rhythms were observed in 67% of individuals, with peak VOD in summer and minimum in winter. Lunar rhythms (29.2 ± 0.7 days) were present in 63 % of individuals (Fig. 5.5B), with peaks around the full moon (Fig. 5.5D). Semilunar rhythms (14.7 ± 0.6 days) were significant in 73 % of individuals, although no significant differences in VOD were observed according to the tidal coefficient partitioning applied in this study (Fig. 5.5B, D). Daily rhythms were observed in 80 % of individuals in spring, 40 % in summer, and 27 % in autumn and winter (Fig. 5.5B). At the group level, the mean VOD activity was significantly nocturnal in summer, and shifted to diurnal in autumn, while no significant day-night differences were detected in spring and winter (Fig. 5.5D). Tidal rhythms were present in spring and summer (13 % and 67 % of rhythmic individuals, respectively), with the lowest VOD observed during high tide in spring and during low tide in summer (Fig. 5.5B, D). As observed for VOA, tidal rhythmicity in VOD disappeared in autumn and winter, and no differences were observed according to tidal cycle phases at these seasons (Fig. 5.5B, C).

Valve opening amplitude (VOA)

A

Method	Range	Analyzed period	Mean rhythmic period	Rhythmic ind. (%)
RAIN	Annual	Full exp.	12 m	80
COSINOR	Lunar	Full exp.	29.4 ± 0.3 d	67
	Semilunar	Full exp.	14.7 ± 0.3 d	60
	Daily	Spring	24.0 ± 0.1 h	93
		Summer	24.0 ± 0.1 h	93
		Autumn	24.1 ± 0.1 h	13
		Winter	24.1 ± 0.1 h	7
	Tidal	Spring	12.5 ± 0.1 h	60
		Summer	12.4 ± 0.1 h	47
		Autumn	/	/
		Winter	/	/



Valve opening duration (VOD)

B

Method	Range	Analyzed period	Mean rhythmic period	Rhythmic ind. (%)
RAIN	Annual	Full exp.	12 m	67
COSINOR	Lunar	Full exp.	29.2 ± 0.7 d	63
	Semilunar	Full exp.	14.7 ± 0.6 d	73
	Daily	Spring	24.1 ± 0.4 h	80
		Summer	24.3 ± 0.2 h	40
		Autumn	25.8 ± 1.0 h	27
		Winter	24.7 ± 0.7 h	27
	Tidal	Spring	12.7 ± 0.6 h	13
		Summer	12.5 ± 0.1 h	67
		Autumn	/	/
		Winter	/	/

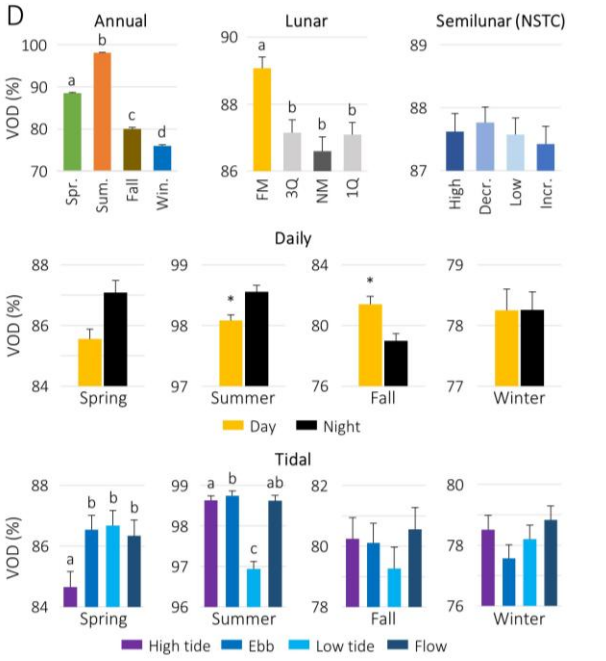


Figure 5.5. Biological rhythms of *Ostrea edulis* valve behavior. (A-B) Results of chronobiological analysis on (A) the individual valve amplitude dataset (VOA, %, n=15) and (B) the individual valve opening duration dataset (VOD, %, n=15), testing annual, lunar and semilunar rhythms during the field experiment, and daily and tidal rhythms during four seasons: spring (20/03/2023 to 20/06/2023), summer (21/06/2023 to

22/09/2023), autumn (23/09/2023 to 21/12/2023) and winter (22/12/2023 to 19/03/2024). (C-D) Mean VOA (C) and VOD (D) segmented according to geophysical cycles' phases (n = 15). The annual histogram displays the mean VOA and VOD values by season. The lunar histogram displays the mean VOA and VOD by moon phases (FM = Full Moon, 3Q = 3rd Quarter, NM = New Moon, and 1Q = 1st Quarter). The semilunar histogram (NSTC = neap-spring tidal cycle) displays the mean VOA and VOD across the tidal coefficient categories (high, decreasing, low, and increasing). Daily histograms display the mean VOA and VOD values during both day and night periods. Tidal histograms show the mean VOA and VOD across to the tide phase (high tide, ebb, low tide, and flow).

Annual rhythms of *Ostrea edulis* gene expression

Annual expression profiles and RAIN analyses of 13 genes involved in circadian clock function (*OeClock*, *OeBmal1*, *OePer*, *OeTim1*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeShag*, *OeCwo*), metabolic regulation (*OeNampt*), melatonin synthesis (*OeHiomt*), and photoreception (*OeCry1*, *OeOpn4*) were assessed in gills and labial palps sampled monthly from July 2023 to July 2024 (Figure 5.6).

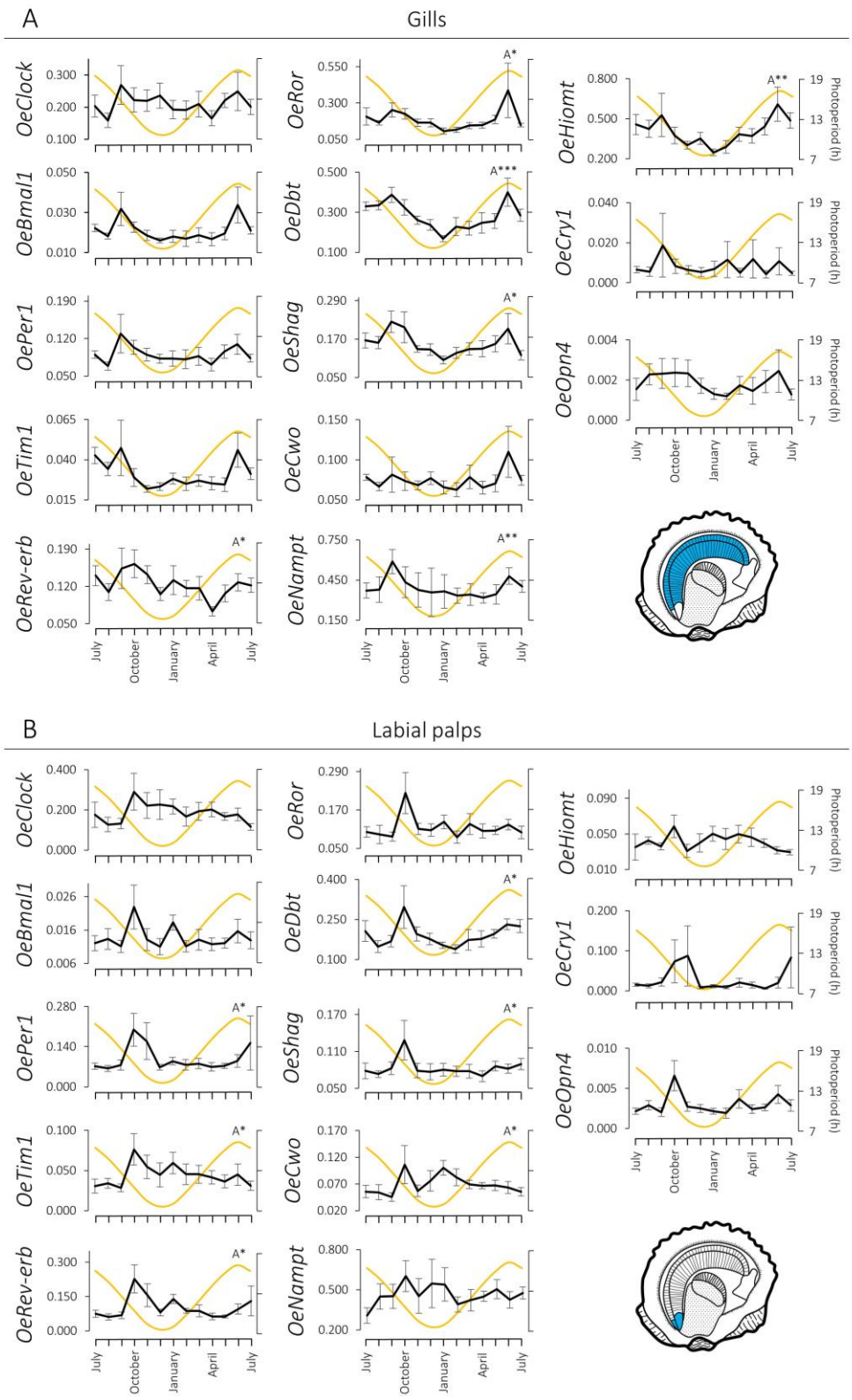

A
Gills
OeClock
OeBmal1
OePer1
OeTim1
OeRev-erb
OeRor
OeDbt
OeShag
OeCwo
OeNampt
OeHiomt
OeCry1
OeOpn4
A*
A**
A***
Photoperiod (h)
July
October
January
April
July
B
Labial palps

Figure 5.6. Annual profiles of expression of genes involved in the clock machinery of *O. edulis* in gills and labial palps in the field. Relative mRNA levels (mean ± SE, n = 8–10 oysters per time point) of nine core circadian clock and clock-related genes (*OeClock*, *OeBmal1*, *OePer1*, *OeTim1*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeShag*, *OeCwo*), a clock-controlled metabolic gene (*OeNampt*), a gene involved in melatonin synthesis (*OeHiomt*), and two photoreceptor genes (*OeCry1*, *OeOpn4*) in (A) gill tissues and (B) labial palps during 13 months of the field experiment (monthly sampling around solar noon, July 2023 to July 2024). Photoperiod is shown in yellow. Statistically significant annual rhythms (RAIN algorithm) are noted with asterisks: A* = $p < 0.05$, A** = $p < 0.01$, A*** = $p < 0.001$. Oyster gills and labial palps are colored in blue in the diagrams (oyster diagrams: ©2020, Native Oyster Network - UK & Ireland, Native Oyster Restoration Alliance).

Overall, expression patterns in gill tissue (Fig. 5.6A) showed two annual peaks, one in September 2023 and the other in June 2024. This is clearly noticeable for genes such as *OeBmal1*, *OeTim1*, *OeDbt*, *OeShag*, and *OeHiomt*. Other genes, such as *OePer1* and *OeCry1* showed a single peak in September, while *OeRor* and *OeCwo* peaked in June. The winter months were associated with lower expression levels for several genes, including *OeBmal1*, *OeTim1*, *OeDbt*, *OeShag*, and *OeHiomt*, suggesting seasonal downregulation. Six genes exhibited significant annual rhythmicity: *OeRev-erb*, *OeRor*, *OeDbt*, *OeShag*, *OeNampt*, and *OeHiomt*.
In labial palps (Fig. 5.6B), the annual patterns differ from those observed in gills, highlighting tissue-specific regulation. Notably, most genes showed a peak of expression in October 2023, one month later than in gills. Here, six genes were identified as rhythmic: *OePer1*, *OeTim1*, *OeRev-erb*, *OeDbt*, *OeShag*, and *OeCwo*.

Discussion

This study provides a first comprehensive, long-term analysis of *Ostrea edulis* phenology in its natural environment, based on *in situ* monitoring of valve activity and shell growth over 540 days, combined with monthly gene expression profiling. Our results show that *O. edulis* valve activity exhibits a complex interaction of biological rhythms, including diel (~24 h), tidal (~12.4 h), semilunar (~14.8 d), lunar (~29.5 d), and annual (~365 d) cycles. The annual rhythm was reflected in both behavior and growth, with peaks in valve activity and shell growth during spring and summer, and a marked reduction in winter entering a resting state. Mixed models revealed that the interaction between

photoperiod and temperature was the primary driver of seasonal variation in activity and growth. Additional environmental cues, such as chlorophyll *a* and underwater noise, had secondary but notable effects. At the molecular level, several circadian clock and clock-related genes displayed significant annual oscillations in both gills and labial palps, suggesting the presence of internal timekeeping mechanisms.

Investigation of *Ostrea edulis* phenology in its natural environment

Seasonal patterns in annual behavioral, growth and molecular rhythms in *O. edulis*

Annual rhythms are essential in aligning physiology and behavior with seasonal environmental changes, thereby improving survival and reproductive success (Dunlap et al., 2004). Our study showed that the phenology of *O. edulis* clearly exhibited annual rhythms at the molecular, behavioral, and physiological levels. In this study, we use the term phenology in a broad sense to describe the seasonal organization of behavioral, physiological, and molecular processes in relation to recurring environmental cycles. Accordingly, valve activity, shell growth, and many gene expressions related to the circadian clock machinery and clock outputs peaked during spring and summer and decreased significantly during autumn and winter. A pronounced winter slowdown is a common strategy among marine organisms. For instance, crustaceans such as copepods (Baumgartner & Tarrant, 2017) remain inactive for several months during autumn and winter to cope with unfavorable conditions. Bivalves also reduce activity during short-day seasons, but *O. edulis* in Helgoland displayed a much stronger winter slowdown than *Crassostrea gigas* at southern latitudes (Payton et al., 2017). However, comparable damped activities have been observed in the mussel *Mytilus spp.* in the Arctic during harsh seasons (Le Moal et al., 2023; Tran, Andrade, et al., 2020). It can be hypothesized that the pronounced winter slowdown of *O. edulis* observed in this study would be less significant at lower latitudes. Testing this hypothesis will require comparative studies, both across bivalve species at similar latitudes and within *O. edulis* populations along its distribution range[38]. Controlled laboratory experiments manipulating temperature under constant photoperiod conditions could also help disentangle the respective effects of temperature and seasonal light cues. Our findings also showed that annual rhythms in O. edulis were synchronized with environmental factors, photoperiod-temperature interaction being the strongest predictor of valve activity and shell growth. Photoperiod is the most stable and predictable cue for annual environmental cycles, indirectly shaping

other factors such as temperature and food availability (Visser et al., 2010). Consequently, it is the most reliable time-giver for endogenous circannual clocks, allowing anticipation and synchronization with predictable annual environmental cycles (Helm & Lincoln, 2017; Visser et al., 2010). In O. edulis, it could allow physiology to be adjusted at each time of the year, anticipating energy conservation during cold or food-poor periods and anticipating increased activity in spring and summer to optimize reserve accumulation and gametogenesis. While the photoperiod-synchronized circannual clock would control the annual timing of biological processes, most of physiological processes depend on the ambient temperature since oysters are poikilotherms, emphasing the role of photoperiod-temperature interaction on *O. edulis* behavior and growth observed in this study.

Recent investigations have identified key circadian clock and clock-related genes in *O. edulis* (Le Moal, Payton, Tran, et al., 2025), providing molecular evidence for temporal regulation. The analyzed clock and clock-related genes, are increasingly recognized for their role in longer-term timekeeping (Helm & Lincoln, 2017; Payton et al., 2024). Several core and clock-related genes, such as *OePer1*, *OeTim1*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeShag*, *OeCwo*, *OeHiomt*, and *OeNampt*, exhibited annual rhythmicity in the gills and labial palps of *O. edulis*, suggesting the possible existence of an endogenous circannual timing system involving the circadian clock machinery. Notably, *OeHiomt*, involved in melatonin synthesis, showed an annual rhythm of expression in *O.edulis*. Given melatonin's known role in seasonal timing and reproductive regulation in animals (Foulkes et al., 1997; Ikegami & Yoshimura, 2017; Reiter, 1993), its expression pattern in *O. edulis* suggests a role in photoperiod-driven synchronization of physiology, including seasonal behaviors and physiological functions.

Overall, the alignment of behavioral, physiological, and molecular rhythms, combined with the absence of observed mortality, reflects strong synchronization with environmental cycles and supports that *O. edulis* was healthy and well adapted to its habitat during the experiment in Helgoland.

Daily rhythm in valve activity

Our results showed that *O. edulis* exhibits daily rhythms of valve behavior. The behavioral and molecular daily rhythms of *O. edulis* have been previously observed in laboratory studies under controlled conditions (Le Moal, Tran, et al., 2025), and were found to be maintained under constant light, demonstrating the presence of a functional endogenous circadian clock. These results supported the fact that the daily rhythms

observed in the field reflect the output of an internal anticipation mechanism shaped by evolution and synchronized by daily light-dark cycles, rather than a direct response to environmental signals (Helm et al., 2017). Interestingly, our study showed that *O. edulis'* daily rhythms of valve activity were stronger in spring and summer, and weakened in autumn and winter. Such annual modulations of daily rhythms were previously observed in the oyster *C. gigas* or the noble pen shell *Pinna nobilis*, and suggest a circannual control of the circadian clock (Garcia-March et al., 2016; Mat et al., 2012; Payton et al., 2017). Moreover, diel activity phase in *O. edulis* shifted with the time of year: oysters are nocturnal during long-day seasons and switch to diurnal activity during short-day seasons. Such seasonal phase reversal has also been reported in the oyster *C. gigas* (Mat et al., 2012), as well as in the burbot *Lota lota* (Müller, 1978; Reebs, 2002). However, in *C. gigas*, the opposite trend occurs, suggesting possible temporal niche partitioning between these cohabiting species (Bloch et al., 2017; Kronfeld-Schor & Dayan, 2003; Zwerschke et al., 2017). This daily rhythm flexibility may optimize feeding, energy use, or predator avoidance across seasons.

Rhythms related to lunar cycles

In addition to daily and annual cycles, our study revealed lunar (~29.5 d), semilunar (~14.8 d), and tidal (~12.4 h) rhythmicities. These cycles are well-established in marine environments, often associated with either light variation (from moon phases) or mechanical forces (from tidal changes) (Tessmar-Raible et al., 2011; Tran et al., 2011). In our study, we clearly identified a lunar rhythm (29.5 days) in *O. edulis* valve activity, with peak activity around full moon phases. Similar moonlight-related rhythms occur in bivalves such as *C. gigas* (Payton et al., 2017; Payton & Tran, 2019), *Mytilus spp.* (Le Moal et al., 2023; Tran et al., 2023b), *P. nobilis* (García-March et al., 2008), and in many other marine invertebrates such as the annelid *Platynereis dumerilii* (Andreatta & Tessmar-Raible, 2020; Zantke et al., 2014). These results support the idea that *O. edulis* detected low-intensity moonlight, possibly via specialized photoreceptors (Kronfeld-Schor et al., 2013). Although the mechanisms underlying moonlight perception remain unresolved, photoreceptive molecules such as cryptochromes and opsins have been proposed as potential candidates (Poehn et al., 2022; Zurl et al., 2022), and orthologs of these genes were identified in our previous characterization of the molecular clock and photoreceptive pathways *O. edulis* (Le Moal, Payton, Tran, et al., 2025). Further experiments would be needed to decipher whether the observed lunar rhythm in *O. edulis* is a direct response to moonlight or the result of a synchronized endogenous circalunar clock (Andreatta & Tessmar-Raible, 2020).

In contrast, the semilunar rhythm (14.8 days) and tidal rhythm (12.4 hours) observed in *O. edulis* are more likely linked to mechanical forces, such as tidal amplitude and water pressure changes. First, a tidal rhythm (~12.4 h) was also observed in *O. edulis*, although it was expressed by fewer individuals compared to diel rhythms, and only during spring and summer. This pattern differs from what is commonly observed in other bivalves where tidal rhythms are more consistently and widely expressed across individuals and seasons, such as observed in C. gigas (Payton et al., 2017; Tran et al., 2011; Tran, Perrigault, et al., 2020b) or the intertidal clams *Chione stutchburyi* (Williams et al., 1993) and *Austrovenus stutchburyi* (Williams & Pilditch, 1997). The rarity of tidal rhythms in *O. edulis* in our study could be attributed to the relatively low tidal amplitude (<2 m) at our subtidal study site, which may reduce the strength of tidal cues (pressure and current variations) that may be too subtle to entrain tidal rhythms in all organisms. Nevertheless, the seasonal expression of both tidal and diel rhythms suggests the presence of a plastic clock mechanism, enabling organisms to anticipate predictable environmental fluctuations and optimize their physiological processes accordingly (Naylor, 2010). This interaction between tidal and diel rhythms has been described in other marine invertebrates, such as *C. gigas* bivalves (Mat et al., 2016; Payton et al., 2017; Tran, Perrigault, et al., 2020b) or crustaceans like the isopod *Excirolana chiltoni* (Enright, 1976) or the copepod *Calanus finmarchicus* (Hüppe et al., 2020).

Additionally, the semilunar rhythm reflects the neap-spring tidal cycle, a biweekly mechanical cue that modulates tidal amplitude and thus reinforces the expression of tidal rhythmicity. This semilunar cycle was observed in our study through higher valve amplitude during spring tides (high tidal coefficient) and aligns with findings in other bivalves such as *C. gigas* (Payton et al., 2017; Tran et al., 2011) and *P. nobilis* (García-March et al., 2008, p. 200) or in the isopod *Eurydice pulchra* (Hastings, 1981). To fully understand the endogenous basis of these rhythms, both laboratory and field studies of clock gene expression across tidal cycles are needed to determine whether *O. edulis* tidal rhythms are endogenously generated or purely reactive to environmental cues.

Environmental Drivers and Emerging Stressors

Mixed-effects models revealed that the interaction between photoperiod and temperature was the strongest predictor of annual rhythms in *O. edulis*. Our findings also suggest contributions from additional environmental cues: underwater sound significantly

affected valve opening amplitude, while chlorophyll *a* influenced valve opening duration, likely reflecting adjustments in feeding behavior. Turbidity and salinity, though less influential, showed notable effects as already showed by Bamber (Bamber, 2023) for the salinity. Water level, reflecting tidal cycles, had a minor but noticeable effect despite low tidal amplitude at our subtidal site.

This dependency on environmental synchronization also introduces vulnerability. Firstly, Artificial light at night (ALAN) disrupts natural photic cycles, potentially desynchronizing circadian and seasonal rhythms by interfering with photoperiod perception (Botté, Payton, & Tran, 2023a; Davies & Smyth, 2018; Gaston et al., 2017; Le Moal, Tran, et al., 2025; Marangoni et al., 2022). Among the surfaces exposed to ALAN, coastal areas are strongly impacted (Davies & Smyth, 2018). Concerning *O. edulis'* habitats, i.e. Europe's coastline areas, 54 % are estimated to be light-polluted, with sky brightness increasing by 9.6% per year (Davies et al., 2014; Kyba et al., 2023). Such shifts in light regimes could impair internal clock entrainment in oysters, as observed in *C. gigas* (Botté, Payton, Lefeuvre, et al., 2023; Botté, Payton, & Tran, 2023b, 2023c), and have already been shown to disrupt *O. edulis* daily rhythm (Le Moal, Tran, et al., 2025), potentially leading to altered physiological functions under clock control such as behavior, growth or reproduction[104]. Secondly, climate change could also threaten the fitness of *O. edulis* by decoupling temperature from photoperiod, causing phenological mismatches during critical stages like gametogenesis and larval development (Bradshaw & Holzapfel, 2010; Helm et al., 2013). Also, noise pollution is an emerging anthropogenic stressor that could therefore disrupt natural behavior rhythms (Gigot et al., 2024; Peng et al., 2015; Tidau & Briffa, 2016). In this study we observed that low frequency underwater sound had a significant influence on valve activity in *O. edulis*, even under conditions of relatively low ambient sound intensity and variation at our site. This result corroborates previous studies showing behavioral responses of bivalves species such as *C. gigas* and *M. edulis* to low-frequency vibrations (<1000 Hz) (Charifi et al., 2017; Roberts et al., 2015). Beyond direct behavioral responses, sound may serve as a mechanical *zeitgeber* for tidal rhythms (Sharma & Chandrashekaran, 2005). However, while the long-term effects of acoustic disturbance on O. edulis remain to be deciphered, our findings suggest that noise exposure could chronically disrupt their behavior, potentially impacting individual fitness and population dynamics (Peng et al., 2015; Tidau & Briffa, 2016). Finally, anthropogenic activities can alter salinity through precipitation changes, freshwater runoff, or

wastewater discharge (Röthig et al., 2023), and increase turbidity through dredging and bottom trawling (Palanques et al., 2001) or through sediment extraction or discard. In our study, we showed that both salinity and turbidity had significant effects on valve behavior in *O. edulis*, suggesting that shifts in salinity could disrupt osmoregulation (Pourmozaffar et al., 2020; Shumway, 1977), while increased turbidity may impair gill function and feeding efficiency (Tuttle-Raycraft & Ackerman, 2019). This highlights the sensitivity of oyster to environmental degradation and points to potential risks for population stability under increasing anthropogenic pressures.

Conservation and Management Considerations

These findings have important implications for conservation and restoration strategies targeting *O. edulis* reefs. The demonstration of both environmental synchronization in the field and endogenous rhythmicity indicates that *O. edulis* is evolutionarily adapted to its habitat, supporting the potential for sustainable oyster reef habitat restoration. However, the reliance on environmental cues also highlights vulnerability to anthropogenic stressors. Restoration planning should therefore incorporate rhythmic biology into site selection and timing. Locations exposed to artificial light, disrupted tidal regimes, acoustic disturbance, elevated turbidity or thermal instability may hinder proper rhythm entrainment, potentially reducing fitness and increasing vulnerability to stressors, including pathogens like *Bonamia ostreae*, a key driver of population collapse (Culloty & Mulcahy, 2007; Sas et al., 2020). More broadly, the rhythmic parameters identified in this study, such as valve activity patterns, shell growth dynamics, and their synchronization with environmental cycles, could serve as practical indicators for assessing habitat suitability and physiological condition in oyster farming and restoration sites. Monitoring the stability or disruption of these rhythms may help identify environments favorable for oyster performance and long-term resilience.

Conclusion

This study reveals that *O. edulis* exhibits multiple synchronized biological rhythms, including daily, tidal, lunar, semilunar, and annual cycles, across molecular, behavioral, and physiological levels in its natural environment. Valve behavior and growth are mainly driven by photoperiod and temperature, two key environmental cues, with additional modulation by water level, chlorophyll *a* concentration (used here as a proxy

for phytoplankton density), ambient sound, turbitidy and salinity. The alignment between internal clocks and environmental cycles, along with the absence of mortality over 540 days, suggests that the oysters were well synchronized and healthy. While this synchronization with various environmental factors enhances ecological adaptability, it also raises vulnerability to anthropogenic stressors like light, noise, and pollution. Understanding *O. edulis* rhythms offers valuable insights for monitoring health, resilience, and habitat suitability, supporting adaptive conservation and restoration in a rapidly changing coastal environment. Future research should focus on assessing the resilience of these rhythms under disturbed conditions to better support conservation and restoration of *O. edulis* into changing coastal ecosystems.

Acknowledgments

A. Le Moal was supported by a grant awarded to B. Meyer and B. Pogoda via AWI Inspires - International Science Program for Integrative Research in Earth Systems. The study contributes to the Helmholtz Research Program “Changing Earth-Sustaining our future” of the research field Earth and Environment of the Helmholtz Association, Topic 6, Subtopic 6.1. and 6.2 as well as Topic 4, Suptopic 4.2. The project was supported by the “PHC PROCOPE” program via the project “OysterClock“ (project-ID: 57701739), funded by the French Ministry for Europe and Foreign Affairs, the French Ministry for Higher Education and Research and the German Academic Exchange Service (DAAD). The was also supported by the French National Research Agency (ANR), LUCIOLE project (ANR-22-CE34- 0010-01).
We thank Tristan Hugh-Jones from ROSSMORE Oysters Ltd., for the oysters and his assistance in the reception of oysters on Helgoland. We are grateful to the RESTORE and PROCEED projects teams and the Helgoland Oyster Hatchery for their support during oyster reception, disinfection, and experimental setup, particularly Bérenger Colsoul, Nicolás Araujo Piñeiro, Martin Sackmann, Henning Hoffmann, Nick Mackay-Roberts and Lennard Klingforth. Special thanks to Celina Behrendt for her help during oyster disinfection and during the design and the construction of the lander frames. We thank the AWI Centre for Scientific Diving (CSD) for their essential work during deployment and monthly sampling at “Margate”, especially Philipp Fischer, Markus Brand, Laura Eickelmann and Miriam Lienkämper, and all other divers involved. We also thank Anna Bergmann and Uwe Nettelmann for their assistance in general logistical support in the Helgoland laboratory. We thank Lukas Hüppe for his help in setting up

the light sensor and processing the light data. We thank Kerstin Korte for her laboratory support in Bremerhaven. Finally, we thank Kristin Tessmar-Raible and Stéphane Pouvreau for their valuable discussions throughout the project.

Data availability

The datasets used and analysed during the current study (individual hourly valve behavior data (VOA and VOD), temperature and underwater noise recordings) are publicly available in PANGAEA® - Data Publisher for Earth & Environmental Science (https://doi.pangaea.de/10.1594/PANGAEA.983652)(Le Moal, Payton, Geerebaert, et al., 2025).

References

Airoldi, L., Balata, D., & Beck, M. W. (2008). The Gray Zone: Relationships between habitat loss and marine diversity and their applications in conservation. *Journal of Experimental Marine Biology and Ecology, Marine Ecology: A Tribute to the Life and Work of John S. Gray*, *366*(1), 8–15. https://doi.org/10.1016/j.jembe.2008.07.034

Airoldi, L., & Beck, M. W. (2007). Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology*, *45*, 345–405.

Andreatta, G., & Tessmar-Raible, K. (2020). The Still Dark Side of the Moon: Molecular Mechanisms of Lunar-Controlled Rhythms and Clocks. *Journal of Molecular Biology, Circadian Regulation: From Molecules to Physiology*, *432*(12), 3525–3546. https://doi.org/10.1016/j.jmb.2020.03.009

Bamber, S. D. (2023). Valve gaping behaviour in the European oyster (*Ostrea edulis*) in response to changes in light intensity when combined with variations in salinity and seawater temperature. *Journal of Experimental Marine Biology and Ecology*, *568*, 151943. https://doi.org/10.1016/j.jembe.2023.151943

Baschek, B., Schroeder, F., Brix, H., Riethmüller, R., Badewien, T. H., Breitbach, G., Brügge, B., Colijn, F., Doerffer, R., Eschenbach, C., Friedrich, J., Fischer, P., Garthe, S., Horstmann, J., Krasemann, H., Metfies, K., Merckelbach, L., Ohle, N., Petersen, W., … Ziemer, F. (2017). The Coastal Observing System for Northern and Arctic Seas (COSYNA). *Ocean Science*, *13*(3), 379–410. https://doi.org/10.5194/os-13-379-2017

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Baumgartner, M. F., & Tarrant, A. M. (2017). The Physiology and Ecology of Diapause in Marine Copepods. *Annual Review of Marine Science*, *9*(Volume 9, 2017), 387–411. https://doi.org/10.1146/annurev-marine-010816-060505

Bayne, B. L. (1969). The gregarious behaviour of the larvae of *Ostrea edulis* L. at settlement. *Journal of the Marine Biological Association of the United Kingdom*, *49*(2), 327–356. https://doi.org/10.1017/S0025315400035943

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., Defeo, O., Edgar, G. J., Hancock, B., Kay, M. C., Lenihan, H. S., Luckenbach, M. W., Toropova, C. L., Zhang, G., & Guo, X. (2011). Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *BioScience*, *61*(2), 107–116. https://doi.org/10.1525/bio.2011.61.2.5

Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators: Lessons from diverse organisms. *Nature Reviews Genetics*, *6*(7), 544–556. https://doi.org/10.1038/nrg1633

Benoit, T., Boyé, A., Chevalier, M., Mourguiart, B., Bacher, C., Jardim, V. L., Bizien, A., & Marzloff, M. P. (n.d.). Marine foundation species facilitate biodiversity: Systematic and quantitative syntheses identify trait-based standards to understand seascape functionality. *Biological Reviews*, *n/a*(n/a). https://doi.org/10.1002/brv.70161

Berghahn, R., & Ruth, M. (2005). The disappearance of oysters from the Wadden Sea: A cautionary tale for no-take zones. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*(1), 91–104. https://doi.org/10.1002/aqc.635

Bingham, C., Arbogast, B., Guillaume, G. C., Lee, J. K., & Halberg, F. (1982). Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*, *9*(4), 397–439.

Bloch, G., Bar-Shai, N., Cytter, Y., & Green, R. (2017). Time is honey: Circadian clocks of bees and flowers and how their interactions may influence ecological communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1734), 20160256. https://doi.org/10.1098/rstb.2016.0256

Botté, A., Payton, L., Lefeuvre, E., & Tran, D. (2023). Is part-night lighting a suitable mitigation strategy to limit Artificial Light at Night effects on the biological rhythm at the behavioral and molecular scales of the oyster *Crassostrea gigas*? *Science of The Total Environment*, *905*, 167052. https://doi.org/10.1016/j.scitotenv.2023.167052

Botté, A., Payton, L., & Tran, D. (2023a). Artificial light at night at environmental intensities disrupts daily rhythm of the oyster *Crassostrea gigas*. *Marine Pollution Bulletin*, *191*, 114850. https://doi.org/10.1016/j.marpolbul.2023.114850

Botté, A., Payton, L., & Tran, D. (2023b). Artificial light at night at environmental intensities disrupts daily rhythm of the oyster *Crassostrea gigas*. *Marine Pollution Bulletin*, *191*, 114850. https://doi.org/10.1016/j.marpolbul.2023.114850

Botté, A., Payton, L., & Tran, D. (2023c). The effects of artificial light at night on behavioral rhythm and related gene expression are wavelength dependent in the oyster *Crassostrea gigas*. *Environmental Science and Pollution Research*, *30*(57), 120375–120386. https://doi.org/10.1007/s11356-023-30793-1

Box, G. E. P., Jenkins, G. M., & Reinsel, G. C. (1994). *Time series analysis: Forecasting and control* (Third edition). Prentice Hall. http://www.gbv.de/dms/bowker/toc/9780130607744.pdf

Bradshaw, W. E., & Holzapfel, C. M. (2010). Light, Time, and the Physiology of Biotic Response to Rapid Climate Change in Animals. *Annual Review of Physiology*, *72*(Volume 72, 2010), 147–166. https://doi.org/10.1146/annurev-physiol-021909-135837

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Cermakian, N., & Sassone-Corsi, P. (2000a). Multilevel regulation of the circadian clock. *Nature Reviews Molecular Cell Biology*, *1*(1), 59–67. https://doi.org/10.1038/35036078

Cermakian, N., & Sassone-Corsi, P. (2000b). Multilevel regulation of the circadian clock. *Nature Reviews Molecular Cell Biology*, *1*(1), 59–67. https://doi.org/10.1038/35036078

Charifi, M., Sow, M., Ciret, P., Benomar, S., & Massabuau, J.-C. (2017). The sense of hearing in the Pacific oyster, Magallana gigas. *PLOS ONE*, *12*(10), e0185353. https://doi.org/10.1371/journal.pone.0185353

Council of the European Union. (1992). *Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of wild Fauna and Flora*. Brussels: European Parliament.

Council of the European Union. (2008). Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 Establishing a Framework for Community Action in the Field of Marine Environmental Policy (Marine Strategy Framework Directive). *Brussels: European Parliament*, 19–40.

Culloty, S. C., & Mulcahy, M. F. (2007). *Bonamia ostreae in the Native Oyster Ostrea edulis*. https://oar.marine.ie/handle/10793/269

Davies, T. W., Duffy, J. P., Bennie, J., & Gaston, K. J. (2014). The nature, extent, and ecological implications of marine light pollution. *Frontiers in Ecology and the Environment*, *12*(6), 347–355. https://doi.org/10.1890/130281

Davies, T. W., & Smyth, T. (2018). Why artificial light at night should be a focus for global change research in the 21st century. *Global Change Biology*, *24*(3), 872–882. https://doi.org/10.1111/gcb.13927

Dunlap, J. C. (1999). Molecular Bases for Circadian Clocks. *Cell*, *96*(2), 271–290. https://doi.org/10.1016/S0092-8674(00)80566-8

Dunlap, J. C., Loros, J. J., & DeCoursey, P. J. (2004). *Chronobiology: Biological timekeeping* (pp. xix, 406). Sinauer Associates.

Eagling, L. E., Ashton, E. C., & Eagle, J. (2015). The incentives of a resource owner: Evidence from a private oyster fishery. *Marine Policy*, *58*, 28–35. https://doi.org/10.1016/j.marpol.2015.04.004

Enright, J. T. (1976). Plasticity in an isopod's clockworks: Shaking shapes form and affects phase and frequency. *Journal of Comparative Physiology*, *107*(1), 13–37. https://doi.org/10.1007/BF00663916

Fischer, P., Brix, H., Baschek, B., Kraberg, A., Brand, M., Cisewski, B., Riethmüller, R., Breitbach, G., Möller, K. O., Gattuso, J.-P., Alliouane, S., van de Poll, W. H., & Witbaard, R. (2020). Operating Cabled Underwater Observatories in Rough Shelf-Sea Environments: A Technological Challenge. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00551

Fischer, P., Lienkämper, M., Brand, M., Bussmann, I., & Brix, H. (2025). Hydrographical time series data of Helgoland, Southern North Sea, 2024 [Dataset]. In *In: Fischer, P et al. (2026): Hydrographical time series data of Helgoland, Southern North Sea, since 2013 [dataset publication series]. PANGAEA, https://doi.org/10.1594/PANGAEA.995221*. PANGAEA. https://doi.org/10.1594/PANGAEA.986331

Fischer, P., Lienkämper, M., Happel, L., Bussmann, I., Eickelmann, L., Anselm, N., Brand, M., & Brix, H. (2024). *Hydrographical time series data of Helgoland, Southern North Sea, 2023* [Dataset]. PANGAEA. https://doi.org/10.1594/PANGAEA.967855

Foulkes, N. S., assone-Corsi, P., Borjigin, J., & Snyder, S. H. (1997). Rhythmic transcription: The molecular basis of circadian melatonin synthesis. *Trends in Neurosciences*, *20*(10), 487–492. https://doi.org/10.1016/S0166-2236(97)01109-0

Fox, J., & Weisberg, S. (2018). An R companion to applied regression. *Sage Publications*.

Garcia-March, J. R., Jiménez, S., Sanchis, M. A., Monleon, S., Lees, J., Surge, D., & Tena-Medialdea, J. (2016). In situ biomonitoring shows seasonal patterns and environmentally mediated gaping activity in the bivalve, Pinna nobilis. *Marine Biology*, *163*(2), 29. https://doi.org/10.1007/s00227-016-2812-3

García-March, J. R., Sanchís Solsona, M. Á., & García-Carrascosa, A. M. (2008). Shell gaping behaviour of Pinna nobilis L., 1758: Circadian and circalunar rhythms revealed by in situ monitoring. *Marine Biology*, *153*(4), 689–698. https://doi.org/10.1007/s00227-007-0842-6

Gaston, K. J., Davies, T. W., Nedelec, S. L., & Holt, L. A. (2017). Impacts of Artificial Light at Night on Biological Timings. *Annual Review of Ecology, Evolution, and Systematics*, *48*(Volume 48, 2017), 49–68. https://doi.org/10.1146/annurev-ecolsys-110316-022745

Gercken, J., & Schmidt, A. (2014). Current status of the European Oyster (*Ostrea edulis*) and possibilities for restoration in the German North Sea. *Bundesamt Für Naturschutz*.

Gigot, M., Tremblay, R., Bonnel, J., Mathias, D., Meziane, T., Chauvaud, L., & Olivier, F. (2024). Noise pollution causes parental stress on marine invertebrates, the Giant scallop example. *Marine Pollution Bulletin*, *203*, 116454. https://doi.org/10.1016/j.marpolbul.2024.116454

Gosling, E. (2003). *Bivalve Molluscs: Biology, Ecology and Culture*. John Wiley & Sons.

Häfker, N. S., Andreatta, G., Manzotti, A., Falciatore, A., Raible, F., & Tessmar-Raible, K. (2023). Rhythms and Clocks in Marine Organisms. *Annual Review of Marine Science*, *15*(Volume 15, 2023), 509–538. https://doi.org/10.1146/annurev-marine-030422-113038

Hastings, M. H. (1981). The entraining effect of turbulence on the circa-tidal activity rhythm and its semi-lunar modulation in Eurydice pulchra. *Journal of the Marine Biological Association of the United Kingdom*, *61*(1), 151–160. https://doi.org/10.1017/S0025315400045987

Helm, B., Ben-Shlomo, R., Sheriff, M. J., Hut, R. A., Foster, R., Barnes, B. M., & Dominoni, D. (2013). Annual rhythms that underlie phenology: Biological time-keeping meets environmental change. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20130016. https://doi.org/10.1098/rspb.2013.0016

Helm, B., & Lincoln, G. A. (2017). Circannual Rhythms Anticipate the Earth's Annual Periodicity. In V. Kumar (Ed.), *Biological Timekeeping: Clocks, Rhythms and Behaviour* (pp. 545–569). Springer India. https://doi.org/10.1007/978-81-322-3688-7_26

Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., & Bloch, G. (2017). Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1734), 20160246. https://doi.org/10.1098/rstb.2016.0246

Hüppe, L., Payton, L., Last, K., Wilcockson, D., Ershova, E., & Meyer, B. (2020). Evidence for oscillating circadian clock genes in the copepod *Calanus finmarchicus* during the summer solstice in the high Arctic. *Biology Letters*, *16*(7), 20200257. https://doi.org/10.1098/rsbl.2020.0257

Ikegami, K., & Yoshimura, T. (2017). Molecular Mechanism Regulating Seasonality. In V. Kumar (Ed.), *Biological Timekeeping: Clocks, Rhythms and Behaviour* (pp. 589–605). Springer India. https://doi.org/10.1007/978-81-322-3688-7_28

Jamil, T., Opdekamp, W., van Diggelen, R., & ter Braak, C. J. F. (2012). Trait-Environment Relationships and Tiered Forward Model Selection in Linear Mixed Models. *International Journal of Ecology*, *2012*(1), 947103. https://doi.org/10.1155/2012/947103

Jamil, T., Ozinga, W. A., Kleyer, M., & ter Braak, C. J. F. (2013). Selecting traits that explain species–environment relationships: A generalized linear mixed model approach. *Journal of Vegetation Science*, *24*(6), 988–1000. https://doi.org/10.1111/j.1654-1103.2012.12036.x

Korringa, P. (1940). *Experiments End Observations on Swarming, Pelagic Life and Setting in the European Flat Oyster, Ostrea Edulis L.: Contribution from the Governmental Institution for Biological Fishery Research*. s.e.

Kronfeld-Schor, N., & Dayan, T. (2003). Partitioning of Time as an Ecological Resource. *Annual Review of Ecology, Evolution, and Systematics*, *34*(Volume 34, 2003), 153–181. https://doi.org/10.1146/annurev.ecolsys.34.011802.132435

Kronfeld-Schor, N., Dominoni, D., de la Iglesia, H., Levy, O., Herzog, E. D., Dayan, T., & Helfrich-Forster, C. (2013). Chronobiology by moonlight. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20123088. https://doi.org/10.1098/rspb.2012.3088

Küfeoğlu, S. (2022). SDG-14: Life Below Water. In S. Küfeoğlu (Ed.), *Emerging Technologies: Value Creation for Sustainable Development* (pp. 453–468). Springer International Publishing. https://doi.org/10.1007/978-3-031-07127-0_16

Kyba, C. C. M., Altıntaş, Y. Ö., Walker, C. E., & Newhouse, M. (2023). Citizen scientists report global rapid reductions in the visibility of stars from 2011 to 2022. *Science (New York, N.Y.)*, *379*(6629), 265–268. https://doi.org/10.1126/science.abq7781

Le Moal, A., Payton, L., Andrade, H., Camus, L., Ballantine, C., Ciret, P., & Tran, D. (2023). Blue mussels' valve behavior exhibits daily and lunar rhythms during the high Arctic polar day. *Marine Biology*, *170*(9), 113. https://doi.org/10.1007/s00227-023-04257-6

Le Moal, A., Payton, L., Geerebaert, Y., Meyer, B., Pogoda, B., & Tran, D. (2025). *Valve behavior of the European flat oyster Ostrea edulis and associated underwater sound and temperature data, recorded during a 18-month in situ experiment in Helgoland, Germany* [Dataset bundled publication]. PANGAEA. https://doi.pangaea.de/10.1594/PANGAEA.983652

Le Moal, A., Payton, L., Tran, D., Daramy, F., Botté, A., Pogoda, B., & Meyer, B. (2025). Identification and expression of circadian clock genes in the European flat oyster *Ostrea edulis*. *Royal Society Open Science*, *12*(12), 250970. https://doi.org/10.1098/rsos.250970

Le Moal, A., Tran, D., Payton, L., Pogoda, B., & Meyer, B. (2025). Characterization of the daily and circadian valve behavior of the European flat oyster *Ostrea edulis*. *Scientific Reports*, *15*(1), 14952. https://doi.org/10.1038/s41598-025-98746-x

Le Moullac, G., Quéau, I., Le Souchu, P., Pouvreau, S., Moal, J., René Le Coz, J., & François Samain, J. (2007). Metabolic adjustments in the oyster *Crassostrea gigas* according to oxygen level and temperature. *Marine Biology Research*, *3*(5), 357–366. https://doi.org/10.1080/17451000701635128

Ledoux, T., Clements, J. C., Comeau, L. A., Cervello, G., Tremblay, R., Olivier, F., Chauvaud, L., Bernier, R. Y., & Lamarre, S. G. (2023). Effects of anthropogenic sounds on the behavior and physiology of the Eastern oyster (Crassostrea virginica). *Frontiers in Marine Science*, *10*. https://doi.org/10.3389/fmars.2023.1104526

Lee, H. Z. L., Davies, I. M., Baxter, J. M., Diele, K., & Sanderson, W. G. (2020). Missing the full story: First estimates of carbon deposition rates for the European flat oyster, *Ostrea edulis*. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2076–2086. https://doi.org/10.1002/aqc.3402

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Maneiro, V., Silva, A., Pazos, A. J., Sánchez, J. L., & Pérez-Parallé, M. L. (2017). Effects of temperature and photoperiod on the conditioning of the flat oyster (*Ostrea edulis* L.) in autumn. *Aquaculture Research*, *48*(8), 4554–4562. https://doi.org/10.1111/are.13280

Marangoni, L. F. B., Davies, T., Smyth, T., Rodríguez, A., Hamann, M., Duarte, C., Pendoley, K., Berge, J., Maggi, E., & Levy, O. (2022). Impacts of artificial light at night in marine ecosystems—A review. *Global Change Biology*, *28*(18), 5346–5367. https://doi.org/10.1111/gcb.16264

Marescaux, J., Falisse, E., Lorquet, J., Van Doninck, K., Beisel, J.-N., & Descy, J.-P. (2016). Assessing filtration rates of exotic bivalves: Dependence on algae concentration and seasonal factors. *Hydrobiologia*, *777*(1), 67–78. https://doi.org/10.1007/s10750-016-2764-0

Mat, A. M., Massabuau, J.-C., Ciret, P., & Tran, D. (2012). Evidence for a Plastic Dual Circadian Rhythm in the Oyster *Crassostrea gigas*. *Chronobiology International*, *29*(7), 857–867. https://doi.org/10.3109/07420528.2012.699126

Mat, A. M., Perrigault, M., Massabuau, J.-C., & Tran, D. (2016). Role and expression of cry1 in the adductor muscle of the oyster *Crassostrea gigas* during daily and tidal valve activity rhythms. *Chronobiology International*, *33*(8), 949–963. https://doi.org/10.1080/07420528.2016.1181645

Merk, V., Colsoul, B., & Pogoda, B. (2020). Return of the native: Survival, growth and condition of European oysters reintroduced to German offshore waters. *Aquatic Conservation Marine and Freshwater Ecosystems*, *30*, 2180–2190. https://doi.org/10.1002/aqc.3426

Mermet, J., Yeung, J., & Naef, F. (2017). Systems Chronobiology: Global Analysis of Gene Regulation in a 24-Hour Periodic World. *Cold Spring Harbor Perspectives in Biology*, *9*(3), a028720. https://doi.org/10.1101/cshperspect.a028720

Möbius, K. A. (1877). *Die Auster und die Austernwirthschaft*. Verlag von Wiegandt, Hemple & Parey.

Müller, K. (1978). The flexibility of the circadian system of fish at different latitudes. *Rhythmic Activity of Fishes*, 91–104.

Naylor, E. (2010). *Chronobiology of Marine Organisms*. Cambridge University Press. https://doi.org/10.1017/CBO9780511803567

Orton, J. H. (1927). Observation and Experiments on Sex-Change in the European Oyster (O. edulis.): Part I. The Change from Female to Male. *Journal of the Marine Biological Association of the United Kingdom*, *14*(4), 967–1045. https://doi.org/10.1017/S0025315400051183

OSPAR. (2009). Background document for *Ostrea edulis* and *Ostrea edulis* beds. *In Biodiversity Series 428. Edited by Jan Haelters and Francis Kerckhof. Paris: OSPAR Commission, 22p.*

Palanques, A., Guillén, J., & Puig, P. (2001). Impact of bottom trawling on water turbidity and muddy sediment of an unfished continental shelf. *Limnology and Oceanography*, *46*(5), 1100–1110. https://doi.org/10.4319/lo.2001.46.5.1100

Partch, C. L., Green, C. B., & Takahashi, J. S. (2014a). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology*, *24*(2), 90–99. https://doi.org/10.1016/j.tcb.2013.07.002

Partch, C. L., Green, C. B., & Takahashi, J. S. (2014b). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology*, *24*(2), 90–99. https://doi.org/10.1016/j.tcb.2013.07.002

Payton, L., Last, K. S., Grigor, J., Noirot, C., Hüppe, L., Conway, D. V. P., Dannemeyer, M., Wilcockson, D., & Meyer, B. (2024). Revealing the profound influence of diapause on gene expression: Insights from the annual transcriptome of the copepod *Calanus finmarchicus*. *Molecular Ecology*, *33*(13), e17425. https://doi.org/10.1111/mec.17425

Payton, L., Sow, M., Massabuau, J.-C., Ciret, P., & Tran, D. (2017). How annual course of photoperiod shapes seasonal behavior of diploid and triploid oysters, *Crassostrea gigas*. *PLOS ONE*, *12*(10), e0185918. https://doi.org/10.1371/journal.pone.0185918

Payton, L., & Tran, D. (2019). Moonlight cycles synchronize oyster behaviour. *Biology Letters*, *15*(1), 20180299. https://doi.org/10.1098/rsbl.2018.0299

Peng, C., Zhao, X., & Liu, G. (2015). Noise in the Sea and Its Impacts on Marine Organisms. *International Journal of Environmental Research and Public Health*, *12*(10), Article 10. https://doi.org/10.3390/ijerph121012304

Perrigault, M., & Tran, D. (2017). Identification of the Molecular Clockwork of the Oyster *Crassostrea gigas*. *PLOS ONE*, *12*(1), e0169790. https://doi.org/10.1371/journal.pone.0169790

Poehn, B., Krishnan, S., Zurl, M., Coric, A., Rokvic, D., Häfker, N. S., Jaenicke, E., Arboleda, E., Orel, L., Raible, F., Wolf, E., & Tessmar-Raible, K. (2022). A Cryptochrome adopts distinct moon- and sunlight states and functions as sun- versus moonlight interpreter in monthly oscillator entrainment. *Nature Communications*, *13*(1), 5220. https://doi.org/10.1038/s41467-022-32562-z

Pogoda, B. (2019). Current Status of European Oyster Decline and Restoration in Germany. *Humanities*, *8*(1), Article 1. https://doi.org/10.3390/h8010009

Pogoda, B., Boudry, P., Bromley, C., Cameron, T. C., Colsoul, B., Donnan, D., Hancock, B., Hugh-Jones, T., Preston, J., Sanderson, W. G., Sas, H., Brown, J., Bonacic, K., von Nordheim, H., & zu Ermgassen, P. S. E. (2020). NORA moving forward: Developing an oyster restoration network in Europe to support the Berlin Oyster Recommendation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2031–2037. https://doi.org/10.1002/aqc.3447

Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., Sanderson, W., & Nordheim, H. von. (2019). The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation: Bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquatic Living Resources*, *32*, 13. https://doi.org/10.1051/alr/2019012

Pogoda, B., Hausen, T., Rothe, M., Bakker, F., Hauser, S., Colsoul, B., Dureuil, M., Krause, J., Heinicke, K., Pusch, C., Eisenbarth, S., Kreutle, A., Peter, C., & Pesch, R. (2023). Come, tell me how you live: Habitat suitability analysis for *Ostrea edulis* restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *33*(7), 678–695. https://doi.org/10.1002/aqc.3928

Pourmozaffar, S., Tamadoni Jahromi, S., Rameshi, H., Sadeghi, A., Bagheri, T., Behzadi, S., Gozari, M., Zahedi, M. R., & Abrari Lazarjani, S. (2020). The role of salinity in physiological responses of bivalves. *Reviews in Aquaculture*, *12*(3), 1548–1566. https://doi.org/10.1111/raq.12397

Preston, J., zu Ermgassen, P., Fabra, M., Lee, H., Rodriguez Perez, A., Hayden-Hughes, M., Colsoul, B., Strand, Å., Ashton, E., Gamble, C., Hancock, B., Sanderson, W., Thurstan, R., Bromley, C., Fariñas-Franco, J., Bonacic, K., Frankic, A., Mcafee, D., & Helmer, L. (2020). *European Native Oyster Habitat Restoration Handbook UK & Ireland*.

R Core Team. (2023). *R: A language and environment for statistical computing*. [Computer software]. Foundation for Statistical Computing. URL https://www.R-project.org/

Reebs, S. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries*, *12*, 349–371.

Reiter, R. J. (1993). The melatonin rhythm: Both a clock and a calendar. *Experientia*, *49*(8), 654–664. https://doi.org/10.1007/BF01923947

Roberts, L., Cheesman, S., Breithaupt, T., & Elliott, M. (2015). Sensitivity of the mussel Mytilus edulis to substrate-borne vibration in relation to anthropogenically generated noise. *Marine Ecology Progress Series*, *538*, 185–195. https://doi.org/10.3354/meps11468

Röthig, T., Trevathan-Tackett, S. M., Voolstra, C. R., Ross, C., Chaffron, S., Durack, P. J., Warmuth, L. M., & Sweet, M. (2023). Human-induced salinity changes impact marine organisms and ecosystems. *Global Change Biology*, *29*(17), 4731–4749. https://doi.org/10.1111/gcb.16859

Sanders, D., Frago, E., Kehoe, R., Patterson, C., & Gaston, K. J. (2021). A meta-analysis of biological impacts of artificial light at night. *Nature Ecology & Evolution*, *5*(1), 74–81. https://doi.org/10.1038/s41559-020-01322-x

Sas, H., Deden, B., Kamermans, P., Ermgassen, P. S. E. zu, Pogoda, B., Preston, J., Helmer, L., Holbrook, Z., Arzul, I., Have, T. van der, Villalba, A., Colsoul, B., Lown, A., Merk, V., Zwerschke, N., & Reuchlin, E. (2020). Bonamia infection in native oysters (*Ostrea edulis*) in relation to European restoration projects. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2150–2162. https://doi.org/10.1002/aqc.3430

Scargle, J. D. (1982). Studies in astronomical time series analysis. II. Statistical aspects of spectral analysis of unevenly spaced data. *The Astrophysical Journal*, *263*, 835–853. https://doi.org/10.1086/160554

Sharma, V. K., & Chandrashekaran, M. K. (2005). Zeitgebers (time cues) for biological clocks. *Current Science*, *89*(7), 1136–1146.

Shumway, S. E. (1977). Effect of salinity fluctuation on the osmotic pressure and Na+, Ca2+ and Mg2+ ion concentrations in the hemolymph of bivalve molluscs. *Marine Biology*, *41*(2), 153–177. https://doi.org/10.1007/BF00394023

Teschke, K., Karez, R., Schubert, P. R., & Beermann, J. (2020). Colonisation success of introduced oysters is driven by wave-related exposure. *Biological Invasions*, *22*(7), 2121–2127. https://doi.org/10.1007/s10530-020-02246-0

Tessmar-Raible, K., Raible, F., & Arboleda, E. (2011). Another place, another timer: Marine species and the rhythms of life. *BioEssays*, *33*(3), 165–172. https://doi.org/10.1002/bies.201000096

Thaben, P. F., & Westermark, P. O. (2014). Detecting Rhythms in Time Series with RAIN. *Journal of Biological Rhythms*, *29*(6), 391–400. https://doi.org/10.1177/0748730414553029

Thomas, S., Collins, K., Hauton, C., & Jensen, A. (2022a). A Review of the Ecosystem Services Provided by the Native Oyster (*Ostrea edulis*): Implications for Restoration. *IOP Conference Series: Materials Science and Engineering*, *1245*(1), 012010. https://doi.org/10.1088/1757-899X/1245/1/012010

Thomas, S., Collins, K., Hauton, C., & Jensen, A. (2022b). A Review of the Ecosystem Services Provided by the Native Oyster (*Ostrea edulis*): Implications for Restoration. *IOP Conference Series: Materials Science and Engineering*, *1245*(1), 012010. https://doi.org/10.1088/1757-899X/1245/1/012010

Thurstan, R. H., McCormick, H., Preston, J., Ashton, E. C., Bennema, F. P., Cetinić, A. B., Brown, J. H., Cameron, T. C., Costa, F. da, Donnan, D., Ewers, C., Fortibuoni, T., Galimany, E., Giovanardi, O., Grancher, R., Grech, D., Hayden-Hughes, M., Helmer, L., Jensen, K. T., … Ermgassen, P. S. zu. (2023). *Historical dataset details the distribution, extent and form of lost Ostrea edulis reef ecosystems.* https://ecoevorxiv.org/repository/view/6359/

Tidau, S., & Briffa, M. (2016). Review on behavioral impacts of aquatic noise on crustaceans. *Proceedings of Meetings on Acoustics*, *27*(1), 010028. https://doi.org/10.1121/2.0000302

Tonk, L., Witbaard, R., Dalen, P. van, Cheng, C. H., & Kamermans, P. (2023). Applicability of the gape monitor to study flat oyster (*Ostrea edulis*) feeding behaviour. *Aquatic Living Resources*, *36*(6), 6. https://doi.org/10.1051/alr/2022021

Tran, D., Andrade, H., Camus, L., Leopold, P., Ballantine, C., Berge, J., Durier, G., Sow, M., & Ciret, P. (2023a). Evidence of separate influence of moon and sun on light synchronization of mussel's daily rhythm during the polar night. *iScience*, *26*(3). https://doi.org/10.1016/j.isci.2023.106168

Tran, D., Andrade, H., Camus, L., Leopold, P., Ballantine, C., Berge, J., Durier, G., Sow, M., & Ciret, P. (2023b). Evidence of separate influence of moon and sun on light synchronization of mussel's daily rhythm during the polar night. *iScience*, *26*(3). https://doi.org/10.1016/j.isci.2023.106168

Tran, D., Andrade, H., Durier, G., Ciret, P., Leopold, P., Sow, M., Ballantine, C., Camus, L., Berge, J., & Perrigault, M. (2020). Growth and behaviour of blue mussels, a re-emerging polar resident, follow a strong annual rhythm shaped by the extreme high Arctic light regime. *Royal Society Open Science*, *7*(10), 200889. https://doi.org/10.1098/rsos.200889

Tran, D., Nadau, A., Durrieu, G., Ciret, P., Parisot, J.-P., & Massabuau, J.-C. (2011). Field Chronobiology of a Molluscan Bivalve: How the Moon and Sun Cycles Interact to Drive Oyster Activity Rhythms. *Chronobiology International*, *28*(4), 307–317. https://doi.org/10.3109/07420528.2011.565897

Tran, D., Perrigault, M., Ciret, P., & Payton, L. (2020a). Bivalve mollusc circadian clock genes can run at tidal frequency. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192440. https://doi.org/10.1098/rspb.2019.2440

Tran, D., Perrigault, M., Ciret, P., & Payton, L. (2020b). Bivalve mollusc circadian clock genes can run at tidal frequency. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192440. https://doi.org/10.1098/rspb.2019.2440

Tuttle-Raycraft, S., & Ackerman, J. D. (2019). Living the high turbidity life: The effects of total suspended solids, flow, and gill morphology on mussel feeding. *Limnology and Oceanography*, *64*(6), 2526–2537. https://doi.org/10.1002/lno.11202

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), research0034.1. https://doi.org/10.1186/gb-2002-3-7-research0034

Visser, M. E., Caro, S. P., van Oers, K., Schaper, S. V., & Helm, B. (2010). Phenology, seasonal timing and circannual rhythms: Towards a unified framework. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3113–3127. https://doi.org/10.1098/rstb.2010.0111

Waltham, N. J., Elliott, M., Lee, S. Y., Lovelock, C., Duarte, C. M., Buelow, C., Simenstad, C., Nagelkerken, I., Claassens, L., Wen, C. K.-C., Barletta, M., Connolly, R. M., Gillies, C., Mitsch, W. J., Ogburn, M. B., Purandare, J., Possingham, H., & Sheaves, M. (2020). UN Decade on Ecosystem Restoration 2021–2030—What Chance for Success in Restoring Coastal Ecosystems? *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00071

Williams, B. G., Palmer, J. D., & Hutchinson, D. N. (1993). Comparative studies of tidal rhythms XIII. Is a clam clock similar to those of other intertidal animals? *Marine Behaviour and Physiology*, *24*(1), 1–14. https://doi.org/10.1080/10236249309378873

Williams, B. G., & Pilditch, C. A. (1997). The Entrainment of Persistent Tidal Rhythmicity in a Filter-Feeding Bivalve Using Cycles of Food Availability. *Journal of Biological Rhythms*, *12*(2), 173–181. https://doi.org/10.1177/074873049701200208

Yonge, C. M. (1926). Structure and Physiology of the Organs of Feeding and Digestion in *Ostrea edulis*. *Journal of the Marine Biological Association of the United Kingdom*, *14*(2), 295–386. https://doi.org/10.1017/S002531540000789X

Young, S. R. (1979). Effect of temperature change on the metabolic rate of an antarctic mite. *Journal of Comparative Physiology*, *131*(4), 341–346. https://doi.org/10.1007/BF00688809

Zantke, J., Oberlerchner, H., & Tessmar-Raible, K. (2014). Circadian and Circalunar Clock Interactions and the Impact of Light in Platynereis dumerilii. In H. Numata & B. Helm (Eds.), *Annual, Lunar, and Tidal Clocks: Patterns and Mechanisms of Nature's Enigmatic Rhythms* (pp. 143–162). Springer Japan. https://doi.org/10.1007/978-4-431-55261-1_8

Zhu, Y., Yu, Z., Liao, K., Zhang, L., Ran, Z., Xu, J., & Yan, X. (2022). Melatonin in razor clam *Sinonovacula constricta*: Examination of metabolic pathways, tissue distribution, and daily rhythms. *Aquaculture*, *560*, 738548. https://doi.org/10.1016/j.aquaculture.2022.738548

zu Ermgassen, P., Hancock, B., B, D., Greene, J., E, S., Spalding, M., & R., B. (2016a). *Setting Objectives for Oyster Habitat Restoration Using Ecosystem Services: A Manager's Guide.*

zu Ermgassen, P., Hancock, B., B, D., Greene, J., E, S., Spalding, M., & R., B. (2016b). *Setting Objectives for Oyster Habitat Restoration Using Ecosystem Services: A Manager's Guide.*

zu Ermgassen, P., McCormick, H., Debney, A., Fariñas-Franco, J. M., Gamble, C., Gillies, C., Hancock, B., Laugen, A. T., Pouvreau, S., Preston, J., Sanderson, W. G., Strand, Å., & Thurstan, R. H. (2025). European Native Oyster Reef Ecosystems Are Universally Collapsed. *Conservation Letters*, *18*(1), e13068. https://doi.org/10.1111/conl.13068

zu Ermgassen, P. S. E., Thurstan, R. H., Corrales, J., Alleway, H., Carranza, A., Dankers, N., DeAngelis, B., Hancock, B., Kent, F., McLeod, I., Pogoda, B., Liu, Q., & Sanderson, W. G. (2020). The benefits of bivalve reef restoration: A global synthesis of underrepresented species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2050–2065. https://doi.org/10.1002/aqc.3410

Zurl, M., Poehn, B., Rieger, D., Krishnan, S., Rokvic, D., Veedin Rajan, V. B., Gerrard, E., Schlichting, M., Orel, L., Ćorić, A., Lucas, R. J., Wolf, E., Helfrich-Förster, C., Raible, F., & Tessmar-Raible, K. (2022). Two light sensors decode moonlight versus sunlight to adjust a plastic circadian/circalunidian clock to moon phase. *Proceedings of the National Academy of Sciences*, *119*(22), e2115725119. https://doi.org/10.1073/pnas.2115725119

Zwerschke, N., Kochmann, J., Ashton, E., Crowe, T., Roberts, D., & O'Connor, N. (2017). Co-occurrence of native *Ostrea edulis* and non-native *Crassostrea gigas* revealed by monitoring of intertidal oyster populations. *Journal of the Marine Biological Association of the United Kingdom*, *98*, 1–10. https://doi.org/10.1017/S0025315417001448

6. Article IV - *Ostrea edulis* valve behavior during spawning events

Spawning to Swarming: valve movement signatures reveal reproductive timing in *Ostrea edulis*

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Submitted in Nature communications

Abstract

Oysters have complex reproductive life cycles, with many species exhibiting hermaphroditism and sequential sex changes. In the European flat oyster (*Ostrea edulis*), a threatened reef-forming species central to restoration efforts, key reproductive processes remain poorly characterized. Here, we used high-frequency non-invasive valvometry to continuously monitor the behavior of individual oysters *in situ* over two consecutive reproduction seasons. We reconstructed the reproductive sequence from fragmented literature, integrating key stages from gamete release to larval swarming and identifying remaining knowledge gaps. We identified and characterized a stereotyped sequence of male and female spawning behaviors, including trans-ctenidial ovulation and incubation. Reproduction events were structured by annual, diel, tidal, and lunar cycles, while temperature primarily modulated incubation duration. We also documented rapid sex changes during the reproduction seasons. Together, these findings establish an integrative behavioral framework of *O. edulis* reproduction under natural conditions, providing new insights into the timing and coordination of reproductive behavior, with implications for ecology, ecosystem restoration and aquaculture.

Keywords: *Ostrea edulis*, ovulation, spawning, larvae incubation, sex differentiation, valve behavior, oyster

Introduction

The timing of gamete release is a critical life-history event in aquatic animals, particularly for species with annual reproduction cycles. Reproductive success depends on the precise synchronization of gamete release between individuals to maximize fertilization in open water (Levitan & Petersen, 1995; Mercier & Hamel, 2010; Yund, 2000). While most studies have emphasized long-term reproductive processes, such as gametogenesis, larval development, and life-history evolution (Fournier et al., 2012; Henshaw et al., 2014; Starr et al., 1990), short-term events like spawning and fertilization timing remain less documented due to the difficulty of observing them at high temporal resolution and under natural conditions. Reproductive synchronization in marine invertebrates is generally mediated by endogenous biological clocks that are entrained by external environmental cues, known as *zeitgebers* (time-givers) (Bernard et al., 2016; Mercier & Hamel, 2010; Sáenz de Miera et al., 2014). Among these cues, astronomical cycles play a central role as reliable *zeitgebers,* aligning reproductive timing with predictable environmental conditions (Bernard et al., 2016; Korringa, 1947; Orton, 1927). These predictable cyclic drivers define the temporal framework within which spawning can occur. At the seasonal scale, the annual photoperiod cycle regulates gametogenesis and defines the spawning window, thereby constraining the period during which reproduction events can occur (Fabioux et al., 2005; McClintock & Watts, 1990). Lunar cycles further contribute to this synchronization, with spawning events often reported during spring tides (Bernard et al., 2016; Korringa, 1947; Lubet, 1991; Prytherch, 1929). At a finer scale, diel light-dark and tidal cycles also synchronize spawning events (Bernard et al., 2016). Besides predictable *zeitgebers*, additional environmental factors may act as proximate triggers of spawning. While temperature is a necessary prerequisite for spawning, it is rarely sufficient on its own (Marteil, 1976; Nelson, 1928). Other environmental cues may contribute to triggering spawning, including abrupt changes in temperature and salinity (Marteil, 1976), hydrodynamic conditions such as waves and currents (Lubet, 1991), phytoplankton availability (Starr et al., 1990), and twilight spectral shifts (Sweeney et al., 2011).

Additionally, the presence of sperm in the water column can act as a pheromonal cue for female spawning, suggesting a role for gamete-mediated signaling in synchrony (Bernay et al., 2006; Galtsoff, 1938b; Rice et al., 2002). Despite its ecological relevance, the fine-scale synchronization of spawning remains poorly understood. Improving our understanding of these mechanisms is therefore essential for the conservation and restoration of vulnerable marine invertebrates in the context of accelerating

environmental change, which may alter phenology and disrupt reproductive timing (Parmesan, 2006). Such alterations can lead to temporal mismatches between endogenous biological rhythms and environmental cues, potentially reducing reproductive success (Edwards & Richardson, 2004; Visser & Gienapp, 2019). In particular, emerging evidence suggests that artificial light at night (ALAN) can interfere with light-mediated spawning cues, as demonstrated in corals, further highlighting the sensitivity of reproductive timing to anthropogenic disturbances (Ayalon et al., 2021; Davies et al., 2023).

This study focuses on a key bivalve species, the European flat oyster (Ostrea edulis Linnaeus, 1758), a historically dominant reef-forming species along European coastlines that has suffered severe population declines over the past century due to over exploitation, habitat degradation, biotic interaction imbalance, and the spread of invasive parasites such as Bonamia ostreae (Culloty & Mulcahy, 2007; Pogoda, 2019; Pouvreau et al., 2023; Sas et al., 2020; Thurstan et al., 2023). Now considered regionally extinct or critically endangered across much of its native range (OSPAR, 2009; zu Ermgassen et al., 2020), O. edulis is nonetheless recognized for its ecological importance as a reef builder, enhancing habitat complexity, stabilizing substrates, and supporting biodiversity (Waltham et al., 2020; zu Ermgassen et al., 2016, 2020). The species is now the focus of restoration efforts and reintroduction programs that have gained momentum in recent years (Pogoda et al., 2019, 2020), but their success depends on a deeper understanding of this species' ecology and reproductive biology under natural conditions, knowledge that remains still limited, unlike the well-studied Pacific oyster Crassostrea gigas. A specific trait of O. edulis is that the species is larviparous performing internal fertilization and larval brooding (Bayne, 2017; Colsoul et al., 2021; His et al., 1999). This strategy, shared across the genus *Ostrea*, represents an ancient evolutionary innovation of parental care that distinguishes it from *Crassostrea* genus and other broadcast-spawning oysters (Ó Foighil & Taylor, 2000). Individuals typically mature first as males and may subsequently become functional females, with the ability to change sex multiple times within a single reproduction season (Cole, 1942; Orton, 1927). During spawning, males release sperm aggregates called spermatozeugmata into the water column, which are subsequently filtered by females into the pallial cavity (Suquet et al., 2018). Ovulated oocytes are then transferred from the suprabranchial chamber into the pallial cavity through the gills via trans-ctenidial ovulation (TCO) into the pallial cavity, where fertilization and larval incubation occur before the release of free-swimming larvae during the swarming phase (Bayne, 2017; Galtsoff, 1938a; Ó Foighil & Taylor, 2000). This larviparous strategy is relatively rare among molluscs and enhances larval survival by protecting early developmental stages from predators and unfavorable environmental

conditions (Gray et al., 2022). However, although several components of this reproductive sequence have been previously described, existing knowledge remains fragmented, largely qualitative, and often derived from laboratory studies, hatchery observations, or partial field descriptions. As a result, the complete succession, timing, and coordination of these reproductive processes under natural conditions remain poorly resolved. In particular, the fine-scale synchronization of reproduction events between individuals and with environmental cues remains poorly characterized, as direct observation of these transient phases in the wild is scarce. Much of our knowledge still relies on hatchery studies, invasive techniques, or early field observations on O. edulis (Korringa, 1940, 1947) or related species such as Ostrea virginica (Galtsoff, 1938a, 1938b).

Advances in automated behavioral monitoring offer new opportunities to investigate spawning processes of marine invertebrates directly in the field. Valve activity recording, which tracks the dynamic opening and closing of bivalve shells, is widely recognized as a reliable proxy for bivalve activity, providing insights into key physiological and behavioral processes such as feeding, respiration, and reproduction (Andrade et al., 2016; Bernard et al., 2016; Tonk et al., 2023). Because shell movements are closely tied to gamete release, brooding, and larval release, non-invasive continuous valvometric recordings can reveal the precise timing and characterize spawning events with minimal disturbance to the animals (Bernard et al., 2016; Payton et al., 2017). In this study, we used high-frequency non-invasive (HFNI) valvometry (Andrade et al., 2016; Tran et al., 2023) to monitor the reproductive activity of O. edulis in the field. The study was conducted near the island of Helgoland in the German Bight, a historical natural subtidal habitat of the species (Pogoda, 2019). Over 540 consecutive days, from March 2023 to August 2024, we recorded valve opening amplitude (VOA) at high temporal resolution for 16 oysters across two consecutive spawning seasons, with temperature measured concurrently. This unique dataset provides an unprecedented opportunity to describe shell movement patterns associated with key reproductive processes, including male gamete release, female ovulation, internal fertilization, larval incubation, and swarming. The primary aim of this study is to characterize, through the valve behavior, the fine-scale *in situ* spawning behavior of *O. edulis*, with particular focus on sex-specific activity, incubation duration, and the temporal structure of successive spawning events. We also investigated the occurrence of sex transitions within individuals, quantified the time elapsed between successive sex changes. In addition, we evaluated whether reproduction events were synchronized with environmental and astronomical cues across multiple

temporal scales, including the annual photoperiod cycle, tidal cycles, lunar phases, diel cycles, and the anomalistic lunar cycle (Earth-Moon distance), while assessing the influence of temperature on incubation duration. By combining an extensive synthesis of previous observations in pioneer works with accurate long-term HFNI valvometry recordings, our study provides a new and high-resolution behavioral framework for the reproductive biology of O. edulis, offering critical insights for the timing and management of oyster reef habitat restoration targeting this ecological key species.

Material and methods

Experimental model

All research detailed in this study complied with German law, and the experiment was conducted in accordance with international ethical standards. The regulatory framework for research involving living marine organisms is provided by the Nature Reserve Ordinance *Helgoländer Felssockel* and the management plan for the Fauna-Flora-Habitat area DE-1813-391 Helgoland with *Helgoländer Felssockel*. The experiment was conducted with 16 European flat oysters, *O. edulis* (approximately 8-10 years old), of a mean (± SE) shell length of 76.9 ± 1.1 mm and shell width of 79.1 ± 1.2 mm. In February 2023, oysters were collected from Loch Ryan, southwest Scotland (54°59′10″ N, 5°03′18″ W; Rossmore Oysters Ltd.), a sustainably managed fishery. Oysters were cleaned individually by hand to remove fouling and biofouling, disinfected, and monitored for mortality during quarantine and acclimation for three weeks in outdoor isolated tanks of the Alfred Wegener Institute (AWI)'s Helgoland-Oyster-Nursery (54°10'40" N, 7°53'31" E) under natural light conditions. Individuals were maintained in a continuous flow-through system supplied with natural seawater from the North Sea and fed a microalgae mixture of *Chaetoceros sp.* (CCAP1010/3), *Isochrysis galbana* (CCAP941/3), and *Rhodomonas salina* (CCMP1319), supplied by the AWI Helgoland-Oyster-Hatchery. In March 2023, oysters were transferred to the field.

Experimental setup

The experiment was conducted at AWI´s underwater experimental site Margate near Helgoland, Germany (at "Margate" site, 54° 11′ 35″ N, 7° 52′ 45″ E) over a period of 540 days, from the 11th of March 2023 to the 31st of August 2024 (Le Moal et al., 2026). The oysters were divided in 16 individual chambers, which were positioned in an oyster basket (SEAPA), for a continuous record of valve behavior using a high-frequency non-

invasive (HFNI) valvometer. The oyster basket was attached to a stainless-steel lander with a cubic structure and 838 cm^3 volume, deployed at a 10 m depth. It was equipped with mesh frames on each side to protect the oysters from predators, without obstructing water flow. Water temperature was measured every 10 seconds (ADT7420 sensor, AnalogDevices) near the valvometer. All underwater operations, including lander and oyster basket deployment, valvometer and sensor installation at Margate, as well as maintenance procedures and oyster sampling during the experiment, were conducted by scientific divers from the AWI Centre for Scientific Diving (CSD). The protective mesh frames surrounding the oysters were replaced and cleaned monthly. In addition to the instrumented oysters, supplementary individuals were deployed in separate baskets attached to a nearby lander to allow periodic sampling. On 27 July 2024, a total of 33 oysters were collected during a putative incubation period. These individuals were opened and examined through macroscopic observations, including gonad examination and assessment of larval characteristics (*e.g.*, presence/absence of larvae and brood color: white *vs.* grey). This sampling procedure was designed to provide independent observations of reproductive condition in parallel with the valvometry recordings.
Behavioral and temperature data were transmitted daily to the Helgoland diving center *via* an underwater node unit connected to shore by a submarine cable providing both power supply and internet connection to the valvometer and sensors (Fischer et al., 2020). Mean hourly temperature recorded throughout the experiment are publicly available in PANGAEA® - Data Publisher for Earth & Environmental Science (Le Moal et al., 2025). Lunar phase and Earth-Moon distance data (anomalistic lunar cycle) data were obtained from www.timeanddate.com, and tidal coefficient data for Helgoland were retrieved from https://mareespeche.com.

Recording of valve behavior activity

16 oysters were studied using a high-frequency non-invasive (HFNI) valvometer, allowing for the remote and continuous recording of their valve activity. The valvometer consists of a pair of lightweight electrodes (<100 mg) designed to minimize disturbance of oyster behavior. The electrodes were glued to each shell valve with each electrode linked to the HFNI valvometer by a flexible wire allowing undisturbed oyster valve movement. An electromagnetic field is generated by an emitting electrode, and the signal received by the corresponding receiving electrod varies with the inter-valve distance, allowing the measurement of individual oyster valve activity every 1.6 seconds. Further details are given in Tran et al., 2023, and Le Moal et al., 2023. The daily recordings are processed using Labview 8.0 (National Instrument, Austin, TX, USA).

For analysis, valve opening amplitude (VOA) values were averaged on an hourly basis. Individual VOA of each oyster is reported each hour as a percentage, where 100 % indicates that the valves opened at their maximum amplitude during the whole hour and 0 % indicates that the valves were closed over the entire hour; in between all the values of amplitudes are possible. All individual hourly valve behavior data recorded throughout the experiment are publicly available in PANGAEA® - Data Publisher for Earth & Environmental Science (Le Moal et al., 2025).
The mean hourly VOA is represented as double-plotted heatmaps, where each line represents two days. The mean hourly VOA are represented according to a color scale from 0 % to 100 %, divided into eleven intermediate ranges.

Statistical analyses

For each environmental or astronomical variable (daily cycle, synodic cycle, tidal coefficient, tidal coefficient classes, tidal phases, and anomalistic cycle), observed spawning and larval swarming frequencies were compared with those expected under a uniform distribution across predefined temporal or environmental intervals or categories, using Chi-square goodness-of-fit tests. Because some intervals or categories contained low expected frequencies, p-values were estimated by Monte Carlo simulation (10,000 replicates). When the global test was significant, post-hoc exact binomial tests were performed for each interval or category against the expected uniform frequency, and resulting p-values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) procedure. Statistical significance was set at $P < 0.05$. All statistical analyses were performed in R (version 4.2.2; R Core Team (R Core Team, 2023)).

Results

Initial detection of reproduction periods through long-term valvometry data records

Heatmaps highlight strong seasonal patterns of activity with overall high valve opening amplitudes from March-April to September-October, and a strong decrease of amplitudes from October-November to February-March (Figure 6.1). During months of high activity, distinct multi-day pronounced and sustained decreases in VOA are observed, with values dropping below 50%, appearing in light blue in heatmaps (highlighted by arrows). These events were observed in late June, early July, early August, and mid-

September 2023, and again in mid-June, early and mid-July, and mid-August 2024. Because such prolonged reductions in valve opening occurred between June and September, the period known as the spawning season for *O. edulis* (Korringa, 1952; Yonge, 1960), and were repeatedly observed in both 2023 and 2024, we hypothesized that they corresponded to female incubation periods and therefore conducted a more detailed examination of the raw valvometry records.

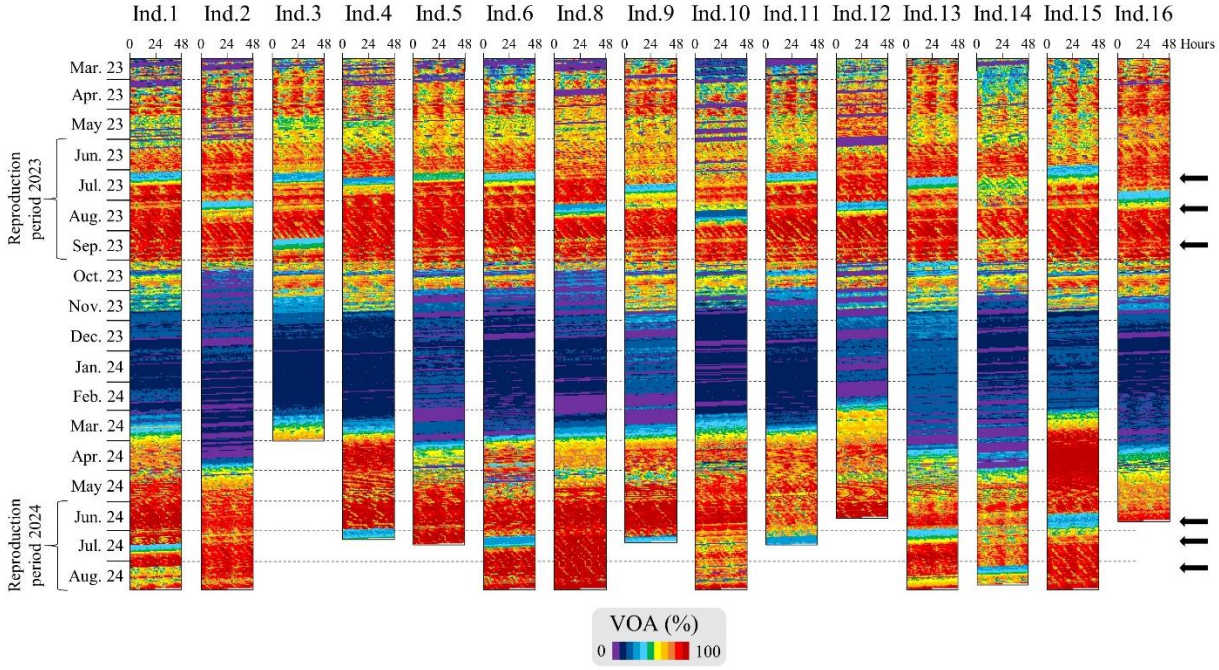


Figure 6.1. Identification of annual reproduction periods in the field from *O. edulis* individuals hourly valve opening amplitude. Double-plotted heatmaps of mean valve opening amplitude (VOA, %) for each oyster (n = 8-15) from 11th March 2023 to 31st August 2024 in Helgoland, Germany. The decreasing number of recorded individuals over the study period reflects technical recording failures. Black arrows on the right indicate the main multi-day decreases in valve opening amplitude identified as putative female incubation periods.

Please note, one oyster (individual 7) died shortly after deployment and was therefore excluded. Technical failures of the set up subsequently reduced the number of continuously recorded individuals from 15 to 14 in March 2024 and from 14 to 8 from June 2024 onwards.

Literature-based reconstruction of the reproductive sequence

To frame the analysis of our results, we reconstructed the sequence of reproduction events in *O. edulis* based on the literature (Table 6.1). Previously described in a

fragmented manner, these processes are here integrated into a unified and coherent framework, highlighting key stages and remaining knowledge gaps.

Table 6.1. Literature-based reconstruction of the reproductive sequence in *O. edulis* and identified knowledge gaps.

Reproductive stage	Description (based on literature)	Key references	Knowledge gaps
Male gamete release	Sperm released into the water column as spermatozeugmata (cohesive bundles of sperm cells that enhance fertilization efficiency); often reported to occur shortly before or concomitantly with female spawning	His et al., 1999; Suquet et al., 2018; Colsoul et al., 2021	Fine-scale timing relative to female reproduction events under natural conditions; degree of synchronization among individuals
Spermatozeugmata capture by females	Spermatozeugmata transported into the pallial cavity through normal filtration currents	Suquet et al., 2018	Direct behavioral evidence and timing relative to male and female gamete release; efficiency and mechanisms of capture *in situ*
Female gamete release / ovulation	Rhythmic adductor muscle contractions associated with valve movements enable release of oocytes into the suprabranchial chamber	Galtsoff, 1938a (*O. virginica*)	Precise behavioral signature; temporal coupling with sperm capture
Trans-ctenidial ovulation (TCO)	Oocytes transferred from suprabranchial chamber to pallial cavity via ctenidial pathways (counter-current transport)	Galtsoff, 1938a (*O. virginica*); Ó Foighil & Taylor, 2000	Timing and dynamics of transfer; direct observation under natural conditions
Fertilization	Internal fertilization occurs within the pallial cavity after mixing of sperm and oocytes	Bayne, 2017	Exact timing and mechanisms of sperm-oocyte interaction
Incubation	Embryos brooded in the mantle cavity for ~1-2 weeks; early reliance on energy reserves contained in yolky eggs, followed by nutrition from food particles captured by the female's feeding currents	Walne, 1974; Chaparro et al., 1993 (*O. chilensis*); Mardones-Toledo et al., 2015 (*O. chilensis*); Bayne, 2017	Continuous behavioral dynamics during incubation; variability under natural environmental conditions

Larval release / swarming	Coordinated valve contractions release veliger larvae into the water column	Erdmann, 1935; Korringa, 1940	Triggers of release; synchronization with environmental cycles; variability among individuals

This synthesis highlights several unresolved aspects, particularly regarding the behavioral timing and coordination of reproduction events under natural conditions, which are addressed below.

Characterization of typical male and female reproductive behaviors

To further characterize the reproduction events suggested by the prolonged reductions in valve opening amplitude identified in Figure 6.1, we conducted a detailed visual inspection of the high-resolution valvometry signals using LabVIEW 8.0 (National Instruments, Austin, TX, USA). In total, 44 spawning events were analyzed (25 in 2023 and 19 in 2024), including 23 female and 21 male events. This analysis, combined with previous bibliographic descriptions of oyster reproductive behavior (Bayne, 2017; Bernard et al., 2016; Galtsoff, 1938a), allowed us to identify recurrent sequences of valve movements associated with male and female reproduction events.

Figure 6.2 summarizes the behavioral patterns identified from these observations. The following nomenclature was adopted to describe the successive reproduction events detected in the valve activity records (Fig. 6.2A): M1, male gamete (spermatozeugmata) release in the water column (male spawning); F1, female spermatozeugmata filtration into the pallial cavity; F2, ovulation in the suprabranchial chamber (female spawning); F3, trans-ctenidial ovulation (TCO); F4, fertilization within the pallial cavity; F5, onset of larval incubation; and F6, larval release (swarming).

Male and female activity profiles display distinct patterns, with females showing a marked reduction in VOA during incubation. In males (Fig. 6.2B, lower panel), spawning is identified by event M1, corresponding to the release of spermatozeugmata into the water column and expressed as a sequence of rapid valve contractions lasting 34.1 ± 3.5 min at a mean frequency of 1.7 ± 0.2 contractions.min^{-1}. In females (Fig. 6.2B, upper panel), spawning follows a stereotyped sequence of successive events. F1 corresponds to a rapid increase of valve opening, interpreted as facilitating the filtration of spermatozeugmata into the pallial cavity. This is followed by F2, a phase of repeated valve contractions corresponding to ovulation and the laying of oocytes into the suprabranchial chamber, lasting 58.5 ± 5.2 min at a mean frequency of 0.5 ± 0.1 contractions.min^{-1}. Event F3 consists of a brief and rapid closure that should correspond

to trans-ctenidial ovulation (TCO), during which oocytes are transferred from the suprabranchial chamber to the pallial cavity. Event F4 is marked by a progressive reopening that may correspond to the beginning of fertilization within the pallial cavity. Event F5 marks the onset of larval incubation, characterized by a pronounced and sustained reduction in VOA of 53.7 ± 11.7% compared to pre-spawning activity (before Event F1), lasting 12.3 ± 0.4 days. This incubation phase can be divided into two successive stages: F5a, a period of relatively stable and low valve opening amplitude lasting 7.6 ± 1.6 days, followed by F5b, during which VOA progressively increases over 4.8 ± 1.6 days, likely reflecting larval development within the pallial cavity. Finally, F6 corresponds to larval release (swarming), characterized by strong and frequent valve contractions lasting 61.2 ± 4.7 min at a mean frequency of 0.9 ± 0.1 contractions.min^{-1}, resulting in the expulsion of veliger larvae into the water column. Raw recordings of individual female incubation periods, male and female spawning sequences, and swarming events are provided in Supplementary Figures S9.11-S9.14. Detailed numerical values are given in Supplementary Tables S9.6-S9.7, while the distributions of event duration, contraction frequency, and ΔVOA (difference between the minimum and maximum VOA recorded during each event) are shown in Supplementary Figure S9.15.

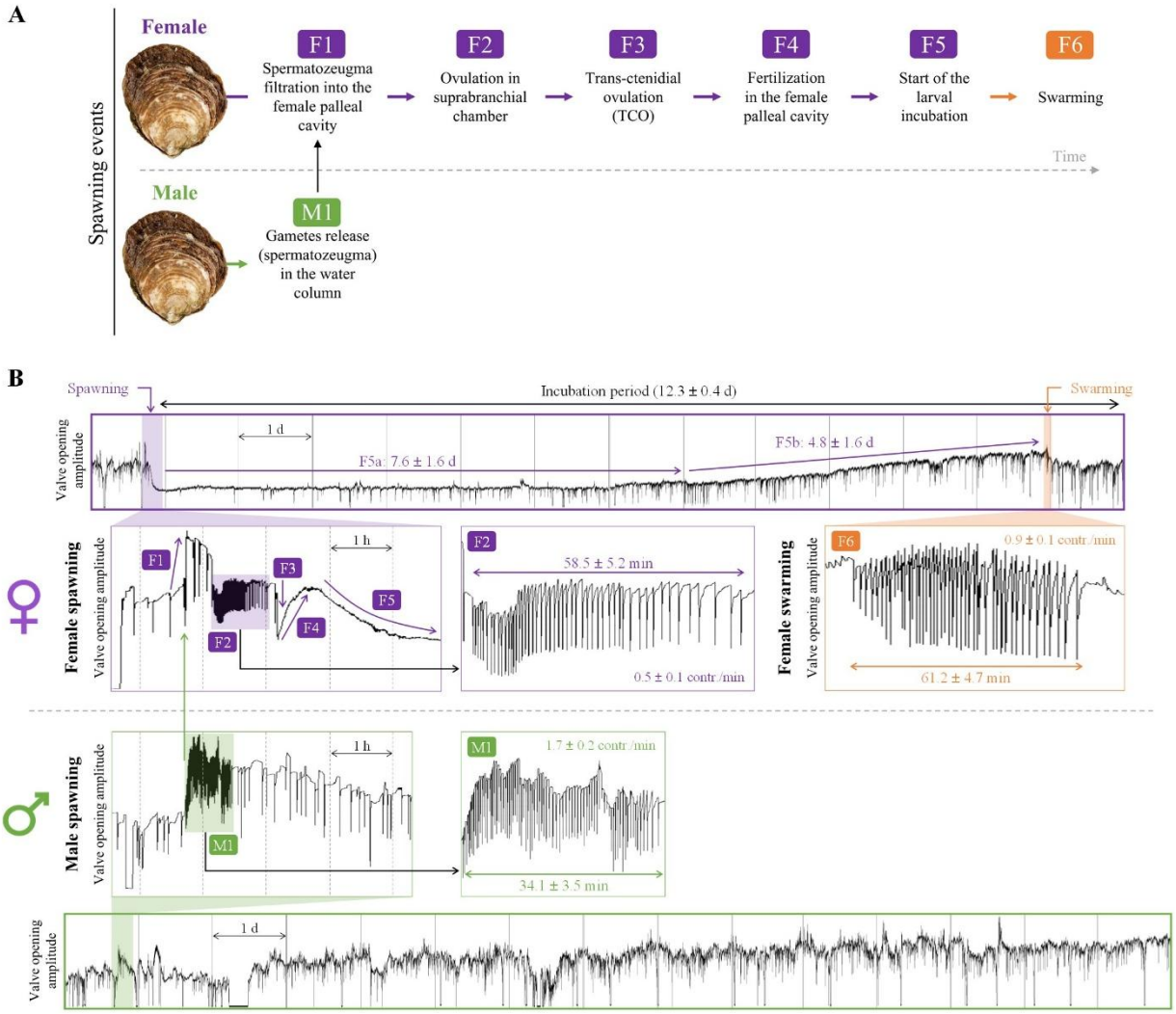


Figure 6.2. Characterization of valve movement signatures associated with the reproductive sequences (events M1, F1-6) in *O. edulis*. (A) Sequence of reproduction events identified in this study, defining the nomenclature to describe male and female reproductive behavior. (B) Typical individual raw valve opening amplitude (VOA) recordings illustrating female and male reproductive sequences over a 14-day period, spanning one day before the onset of female incubation to one day after larval release. The y-axis represents VOA from 0 % to 100 %. Zooms on specific events are also shown. Mean quantitative values across all identified events are expressed as means ± SD.

Individual Dynamics of reproduction events

Individual tracking of reproduction events revealed substantial inter-individual variation in sexual expression (Fig. 6.3), with some oysters reproducing exclusively as females or males, whereas others alternated between sexes within and across reproduction seasons.

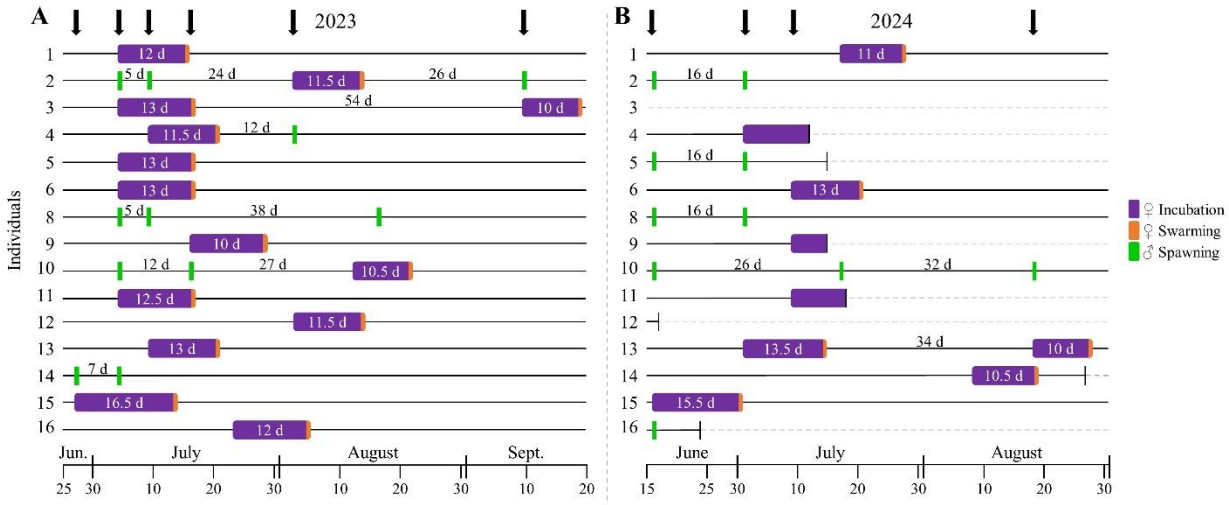


Figure 6.3. *O. edulis* spawning events across two reproduction seasons. Main reproduction events per individual for reproduction seasons 2023 (A) and 2024 (B): Male spawning (green), female incubation including ovulation and fertilization (purple), and larval swarming (orange) are indicated along individual timelines (black lines) including signal losses (grey dashed lines). Black arrows indicate periods of synchronous male and female gamete release across individuals.

This variation was reflected in the individual reproductive trajectories: Some individuals expressed only female (oysters 1, 3, 6, 9, 11, 12, 13, and 15) or male (oyster 8) reproduction events during both 2023 and 2024 spawning seasons, while others alternated between sexes within and across seasons (oysters 2, 4, 5, 10, 14, and 16). Furthermore, the individual number of spawning events per year varies from one (observed only in females, oysters 1, 6, 9, 11, 12, and 15) to four (oyster 2). The shortest observed interval between two male spawning events in one individual was 5 days (oysters 2 and 8 in 2023), while the shortest interval between two female spawning events in one individual was 34 days (oyster 13 in 2024). The shortest observed interval between a female and a male spawning event in a given individual was 12 days (oyster 4 in 2023), while the shortest observed interval between a male and a female spawning event in one individual was 24 days (oyster 2 in 2023). Across both years, male and female spawning events consistently showed strong temporal synchrony (Fig. 6.3), as illustrated by the alignment of events across individuals (highlighted by black arrows;

exact dates of spawning events are provided in Tables S9.6 and S9.7 for female and male events, respectively). On 27th of June 2024, we conducted an independent sampling of 32 additional oysters located near the monitored group to validate reproductive status at the population level. Macroscopic observations (Supplementary Figures S9.16) revealed that 75% of individuals showed no larvae, 16% contained white larvae (early incubation), and 9% contained grey/black larvae (late incubation). These observations are consistent with the valvometric data, with oyster 15 being in incubation at this time, and oysters 2, 5, 8, 10 and 16 having performed a male spawning on 15th of June 2024. Interestingly, in the studied group equipped with valvometry HFNI, oyster 15 was the first female to incubate in both 2023 and 2024, suggesting persistent inter-individual differences in spawning timing. The duration of incubations ranged from to 10 to 16.5 days and showed a progressive reduction from June to September (Fig. 6.3), coinciding with rising seawater temperatures. This relationship was confirmed by a significant negative correlation ($r^2 = 0.824$), indicating a strong effect of temperature on incubation duration (Fig. 6.4).

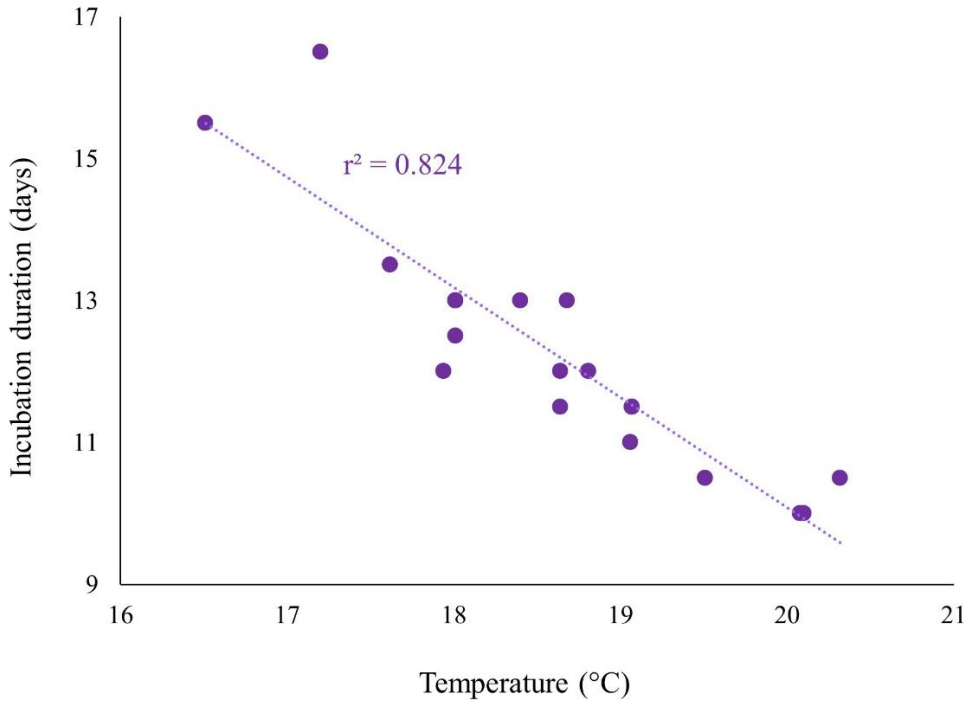


Figure 6.4. **Duration of larval incubation in *O. edulis* in relation to seawater temperature.** Each point represents an individual incubation event (n = 20); the regression line indicates a significant negative correlation between incubation length (days) and temperature (°C). The linear relationship is significant ($p < 0.001$), and its strength is indicated by the coefficient of determination ($r^2 = 0.824$).

Environmental and astronomical timing of spawning events

To explore the influence of astronomical and environmental cycles, the distribution of male and female spawning events as well as larval swarming events were examined. Spawning events occurred predominantly between 15:00 and midnight, with a maximum frequency between 16:00 and 17:00, resulting in a significant non-uniform distribution (Fig. 6.5A). Most spawning events coincided with the warmest period of the day, although daily temperature variation was limited (approximately 18.6-19.0°C). No spawning events were recorded after midnight, when temperature slightly decreased (18.6-18.8°C). Swarming events showed no significant diel pattern, although 85% of the events occurred between 12:00 and midnight. Reproduction events were also examined in relation to the percentage of moon illumination (Fig. 6.5B), related to the synodic cycle (moonlight cycle of ~29.5 d). Spawning events were significantly influenced by this parameter, with higher occurrences around full moon (32%) and, to a lesser extent, a few days after new moon (19%). In contrast, swarming events occurred predominantly during new moon (35%), a pattern that was also statistically significant. In relation to tidal coefficients related to the neap-spring tidal cycle of ~14.8 d and modulated by the anomalistic cycle (Fig. 6.5C), spawning events exhibited a significant, though rather unclear pattern with the majority of events (32 %) observed in intermediate values of tidal coefficient (59-69). For swarming events, a clearer pattern was observed with a majority of events (40 %) in the same tidal coefficient range of 59-69. In relation to the phase of the 12.4 h tidal cycle (Fig. 6.5D), spawning events were significantly more frequent during ebb tide (54%). In contrast, no significant association was detected between swarming events and tidal phases, despite a concentration of 70% of events occurring during high tide and flood phases. Finally, the distribution of reproduction events relative to the Earth-Moon distance (anomalistic lunar cycle of ~29.5 d) was assessed, modulating the intensity of both the synodic cycle and the neap-spring tidal cycle (Fig. 6.5E). Spawning events showed a significant distribution, with no events recorded at perigee (minimal Earth-Moon distance), and most of them occurring at intermediate Earth-Moon distance (between 360 and 380 km) and in apogee (maximal Earth-Moon distance). Swarming events also showed a significant distribution, with a marked peak of occurrence (45%) near apogee.

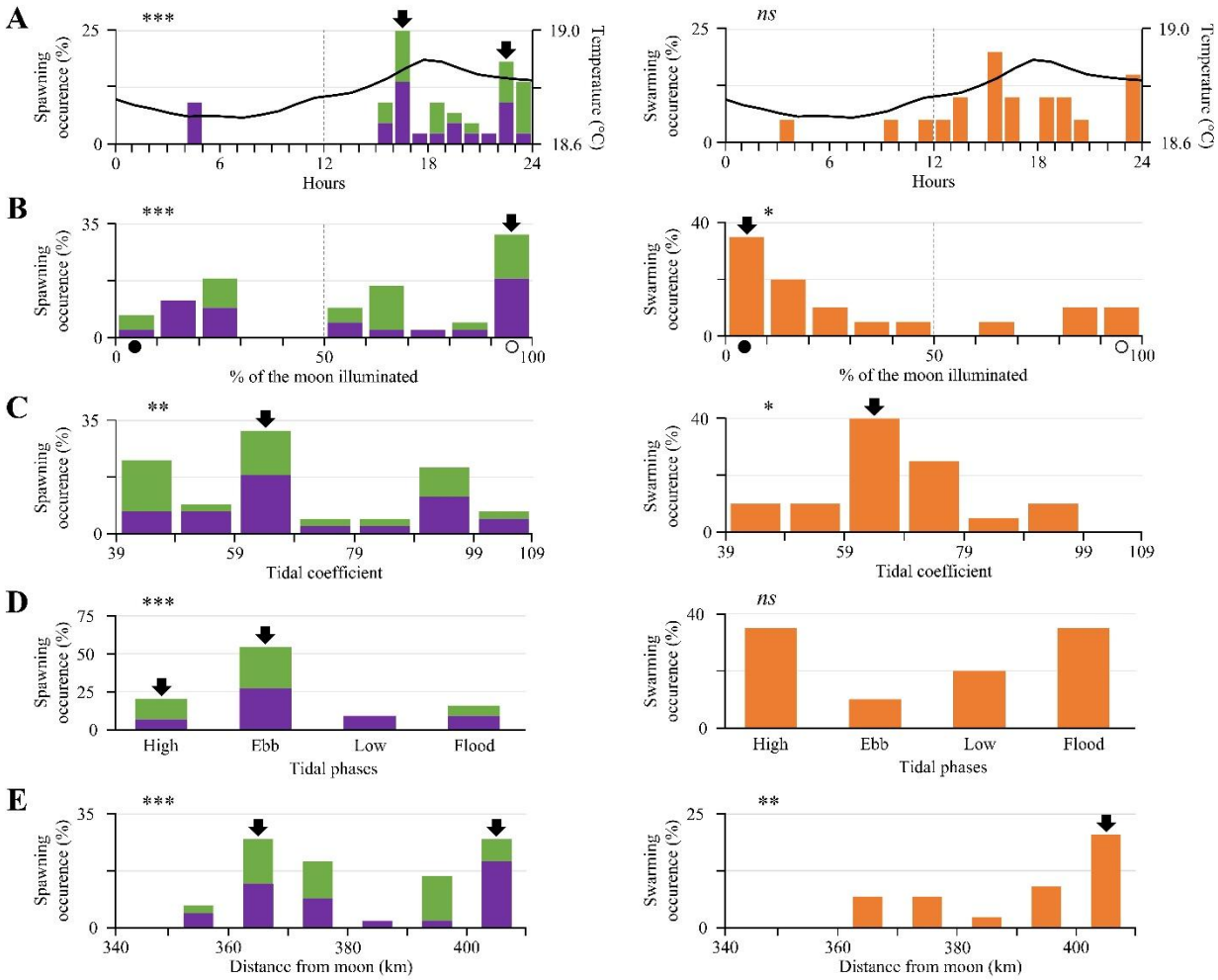


Figure 6.5. Distribution of *O. edulis* reproduction events in relation to astronomical and environmental cycles. Distribution of spawning (male in green, n=21; female in purple, n=23; left panel) and larval swarming (orange, n=20; right panel) events in relation to the daily cycle (A), the synodic cycle (B), the tidal coefficients (C), the tidal cycle (D), and the anomalistic cycle (E). Black lines (A) represent the mean hourly water temperature during summer (21 June - 23 September 2023 and 20 June - 31 August 2024). Significance of deviations from a uniform distribution are indicated as *ns* (not significant), * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Black arrows indicate categories that differed significantly from a uniform distribution in post-hoc binomial tests (FDR-adjusted; $p < 0.05$).

Discussion

Reproduction in the European flat oyster (*O. edulis*) is complex, involving sequential hermaphroditism, internal fertilization, incubation and swarming. Most of the foundational knowledge derives from hatchery observations or descriptive field studies from the early 20th century, often relying on indirect or invasive approaches, including destructive sampling to assess reproductive status (Colsoul et al., 2021; Korringa, 1940, 1947; Orton, 1927). Fine-scale dynamics of reproductive behavior in natural habitats have remained poorly investigated. By using high-frequency non-invasive (HFNI) valvometry, this study is the first to provide a detailed, long-term, *in situ* description of the complete reproductive sequence in *O. edulis*. Based on continuous individual monitoring of valve movements over two consecutive reproduction periods, all phases from gamete release to larval swarming through incubation were identified and documented. These novel results establish a reproducible behavioral framework under natural subtidal conditions, unifying historical and scattered observations with quantitative field-based evidence.

Sex-specific spawning behaviors and incubation dynamics

Male spawning (M1) was characterized by rapid sequences of valve contractions during about half an hour, consistent with the sharp adductor movements described during sperm emission in previous studies (Galtsoff, 1938a; Orton, 1927). Our high-frequency recordings revealed a close temporal association between male spawning and an abrupt and persistent increase in female valve opening amplitude, suggesting tight coordination between sexes. We propose that this marked valve opening enhances ventilation and facilitates the uptake of spermatozeugmata into the pallial cavity (F1), thereby maximizing sperm capture by females before their dispersion and dilution in the water column and optimizing their availability for subsequent internal fertilization. The organization of spermatozoa into spermatozeugmata may also have an adaptive advantage in *O. edulis* by facilitating their capture and retention within the female inhalant cavity, thereby enabling sperm storage until fertilization. A closer investigation of this previously undescribed female response revealed that it started only a few seconds after the onset of male spawning and remained elevated for approximately 28.3 min. Such a rapid and consistent response suggests that, in addition to endogenous reproductive timing and environmental cues, communication between conspecifics may contribute to the remarkable synchronization observed during spawning. Chemical signaling is a plausible mechanism, as waterborne cues are known to mediate

reproductive interactions in a range of marine invertebrates, including bivalves, although other forms of communication, such as social or acoustic cues, have also been proposed (Hamel & Mercier, 1996; Rato et al., 2025; Slattery et al., 1999). In bivalves, chemical cues can influence reproductive processes and are already exploited in hatchery spawning or settlement induction, while the natural mechanisms underlying reproductive synchronization remain poorly characterized (Rato et al., 2025). Future studies should therefore investigate whether chemical or other conspecific cues contribute to the fine-scale coordination of spawning in *O. edulis*.

Based on the characteristic valve-movement patterns identified by HFNI valvometry, we distinguished one male spawning signature (M1) and six female reproductive signatures (F1-F6), corresponding to distinct phases or events associated with spawning, gamete release, swarming, and incubation. Female spawning (F2) was then characterized by typical rhythmic contractions of the adductor muscle lasting about an hour, easily detectable with HFNI valvometry. Interestingly, it occurred after the first step of spermatozeugmata capture (F1), thus leading to a delay between male and female spawning. This spawning pattern closely resembles the "ejection response" described by Orton (Orton, 1927) and later detailed by Galtsoff (Galtsoff, 1938a), described at this time as females respond to the presence of sperm by repeated contractions (Colsoul et al., 2021; His et al., 1999). Similar typical high-frequency contraction patterns have been described as female spawning events in *C. gigas* using HFNI valvometry (Bernard et al., 2016; Payton et al., 2017), lasting 31 ± 8 min and involving rapid adductor contractions at an average frequency of ~1.6 contractions.min^{-1}. Their characteristic rhythm and duration make them easily distinguishable from feeding movements or other routine valve adjustments, providing a clear and distinctive signature of female spawning. While broadly comparable in structure, the spawning sequences described in *C. gigas* more closely resemble the male spawning pattern (M1) observed in *O. edulis*, in terms of both duration (M1: 34.1 ± 3.5 min) and contraction frequency (M1: 1.7 contractions.min^{-1}). In contrast, female spawning in *O. edulis* (F2) differs markedly, with longer durations (F2: 58.5 ± 5.2 min) and lower contraction frequencies (F2: 0.5 contractions.min^{-1}). The similarity between male *O. edulis* and female *C. gigas* signatures may reflect a shared functional constraint associated with gamete release into the water column, as both events involve external gamete expulsion (*C. gigas* performs external fertilization). In contrast, female *O. edulis* must release oocytes into the pallial cavity as part of its larviparous reproductive strategy, which may account for the distinct, longer and less frequent contraction pattern observed during gametes release (F2). Additionally, it is interesting to note that no male spawning behavioral signature have been described in *C. gigas*, suggesting that the expulsion of spermatozoa does not necessitate such specific

valve contractions. The fact that gamete expulsion in males *O. edulis* do require such typical valve movement could be explained by the fact that *O. edulis* expels spermatozeugmata, compact clusters of spermatozoa, larger in size than spermatozoa alone (Suquet et al., 2018). The release of spermatozeugmata may also provide a chemical or behavioral cue to females, potentially contributing to the close temporal coupling between male and female spawning.

Following spawning, females exhibited a closure-reopening sequence (F3/F4). We interpret the closure (F3) as trans-ctenidial ovulation (TCO), during which oocytes are transferred into the inhalant chamber for fertilization and incubation. This transfer of oocytes from the suprabranchial chamber into the pallial cavity is driven by a coordinated mechanism involving adductor contractions and mantle movements. As described by Galtsoff (Galtsoff, 1938a), the closure of the cloaca combined with adductor contraction increases pressure in the suprabranchial chamber, forcing the oocytes through the water tubes and gill ostia. Subsequent reviews have reinforced this view (Ó Foighil & Taylor, 2000). The following reopening (F4) may facilitate mixing between sperm and oocytes, promoting internal fertilization within the pallial cavity (Bayne, 2017), further enhanced by the progressive release of spermatozoa from spermatozeugmata (Suquet et al., 2018).

Following fertilization, females entered a distinct incubation phase (F5), characterized by a sharp decline in valve activity. Valvometry further resolved two successive phases within the incubation phase: an initial period of low and stable amplitude (F5a), followed by a gradual increase until larval release (F5b). This fine-scale resolution provides further details regarding the incubation phase of *O. edulis* previously described at macroscopic scale (Orton, 1927; Spärck, 1925), and echoes observations from the related species *O. chilensis* (Mardones-Toledo et al., 2015). In this study, the incubation was observed to last 12.3 ± 0.4 days depending on temperature, consistent with hatchery observations (Colsoul et al., 2021). During this low-activity period, females nevertheless exhibited intermittent valve contractions, likely serving dual purposes: oxygenating developing embryos (Gray et al., 2022) and expelling dead larvae or pseudofaeces, as observed in other brooding bivalves (Chaparro et al., 2018). Thus, despite the overall low level of valve activity, these micro-closures indicate that incubation is not a state of complete inactivity but rather a finely regulated behavioral state associated with brood care.

Finally, the release of larvae (F6) was marked by strong, repetitive adductor contractions lasting approximately one hour, as for ovulation, but with a higher and more regular frequency, whereas contractions during ovulation tend to become progressively more spaced over time. This behavior corresponds to the classical “swarming” described in

historical observations (Korringa, 1940). Our continuous *in situ* monitoring captures this full sequence (F1-F6), providing the first quantitative linkage, at the individual level, between sperm filtration, ovulation, TCO, fertilization, incubation, and larval release through identifiable valve behaviors.

Synchronization and environmental drivers

At the individual level, spawning was highly synchronized between males and females, but synchronization at the population level was more partial and occurred repeatedly throughout the reproduction season. The largest synchronized events involved 53% of individuals in 2023 and 40% in 2024 (male and female spawning combined). Although a major spawning event occurred in early July, additional spawning events involving fewer individuals were distributed from mid-June to early-September. This pattern contrasts with *C. gigas*, where spawning is more strongly synchronized at the population level, with a large proportion of oysters spawning within the same event and typically a major spawning event early in summer followed by a smaller secondary event later in the season (Bernard et al., 2016; Payton et al., 2017). This partial population-level synchrony suggests that reproductive timing may be structured by cues operating across multiple temporal scales. In temperate marine invertebrates, reproduction can be organized across such multiple temporal scales, from seasonal to diel, lunar or tidal cycles (Mercier & Hamel, 2010; Sweeney et al., 2011). Rather than direct responses to environmental cues, organisms possess a synchronizable endogenous clock allowing them to anticipate the most appropriate environmental conditions for the survival of offspring (Bloch et al., 2013; Helm et al., 2017; Yerushalmi & Green, 2009). The entrainment of endogenous clocks by predictable environmental cycles also allows the synchronization between individuals into a population, particularly crucial in organisms performing external synchronization such as corals (Harrison et al., 1984; Levitan et al., 2011), or the oyster *C. gigas* (Dutertre et al., 2009; Payton et al., 2017). In *O. edulis*, spawning occurred preferentially during the second half of the day and into the early night, with no spawning events recorded during the morning. The broad afternoon-to-night window, together with the concentration of spawning around the daily temperature maximum, argues against a simple direct response to light and instead suggests that diel temperature variation may contribute to the timing of spawning. This interpretation is consistent with the established role of temperature in regulating reproductive activity in *O. edulis* (Colsoul et al., 2021). However, the relatively limited variation in mean daily temperature (18.6-19.0 °C) during summer suggests that this potential thermal contribution should be interpreted with caution. Interestingly, this temporal association differs from that reported in *C. gigas*, in which spawning was observed near the minimum

of the seasonal temperature oscillation, suggesting that the relationship between temperature and spawning timing may be species- or context-dependent (Bernard et al., 2016). Gamete release and fertilization were also preferentially associated with the full moon, consistent with lunar synchronization described by Korringa (Korringa, 1947). However, full-moon periods are normally associated with greater tidal amplitudes, whereas spawning in our study was concentrated at intermediate tidal coefficients (59-69) and preferentially during ebb and high tides. This apparent decoupling could reflect an interaction between the synodic and anomalistic lunar cycles, with spawning occurring more frequently under intermediate or apogean conditions (maximal Earth-Moon distances) may dampen the tidal amplitude associated with the full moon. Such timing could favour gamete dispersal during ebbing while limiting excessive dilution under weaker tidal conditions. From the female perspective, intermediate tidal conditions could also maintain higher local availability of spermatozeugmata, potentially enhancing their uptake during filtration.

Larval swarming showed a partly similar diel pattern, occurring mainly during the second half of the day and into the early night, although this pattern was less pronounced than for spawning. This weaker diel structuring may reflect the more constrained timing of larval release once larvae have reached the developmental stage for emergence. As for spawning, the timing may coincide with the warmer conditions reached later in the day, which could potentially benefit larval activity and survival. In contrast to spawning, swarming was preferentially associated with the new moon and with apogean conditions, but was also concentrated around intermediate tidal coefficients (59-69). This combination of reduced lunar illumination and moderate tidal amplitudes could favor larval dispersal while reducing exposure to visually oriented predators and limiting excessive transport. Together, these patterns suggest that spawning and larval release are not governed by a single environmental cue, but rather by the interaction of diel, lunar, anomalistic and tidal cycles, potentially providing distinct temporal and hydrodynamic conditions for each stage of reproduction.

Beyond these cyclic cues, temperature emerged as an important factor shaping the progression of reproduction once spawning had occurred. Spawning in our study began when water temperature reached approximately 17°C, consistent with previous observations suggesting a minimum temperature requirement of around 15-16°C for gamete emission in *O. edulis*, although this threshold varies among populations and geographic regions (Korringa, 1952; Yonge, 1960). In contrast to this apparent temperature threshold for the onset of spawning, temperature was more clearly associated with the subsequent incubation dynamics. Incubation duration decreased from approximately 16 days in early summer to around 10 days in late summer,

indicating faster progression of larval development under warmer conditions. This pattern is consistent with the temperature dependence of developmental processes reported for laboratory conditions (Yonge, 1960) and extends these observations to natural *in situ* conditions. This temperature dependence is also consistent with patterns observed at the population level. Chapman et al. (Chapman et al., 2021) showed that the seasonal abundance of *O. edulis* larvae in a natural population was strongly associated with cumulative thermal exposure, expressed as temperature sum (the cumulative daily temperature above 7 °C), with peak larval abundance occurring at approximately 617 degree-days. Together with our individual-level observations, these findings suggest that temperature accumulation may contribute to structuring the seasonal progression of larval development and availability in *O. edulis*.

Sex-change dynamics

This study also provides behavioral evidence of sex changes in *O. edulis*. The species is a rhythmically consecutive hermaphrodite (Marteil, 1976), but previous reports relied primarily on histological inference rather than continuous behavioral observation (Maneiro et al., 2017). Here, several individuals were observed switching from male to female or from female to male during the reproduction season, whereas others remained in the same sex throughout the study. Most individuals switched sex only once, while one individual (oyster 2) exhibited the highest number of sex changes, switching from male to female and back to male within the same reproduction season. Male-to-female transition averaged ~25.5 days, while female-to-male transitions occurred more rapidly (~19 days), extending previous observations of sex change dynamics in *O. edulis* (Orton, 1927; Yonge, 1960). Histological studies suggest that incomplete resorption of gametes allows male and female cells to coexist in the gonad, enabling rapid reversals. Notably, the longer duration of the male-to-female transition compared to the female-to-male transition may reflect the higher physiological investment and energy cost required for female function, particularly in preparation for incubation, which involves gametogenesis, internal fertilization, and a prolonged incubation phase. This is in accordance with the much longer duration observed between two female events in a given individual (54 days), compare to the one between two male events (5 days). This sexual plasticity has important ecological implications. At the population level, it buffers against mate limitation, which is critical for small or recovering populations. The ability of individuals to invert sex rapidly ensures that fertilization opportunities are maintained even when local sex ratios are skewed. For hatchery or restoration programs, this emphasizes the need to account for dynamic sex ratios rather than assuming fixed male-

to-female proportions, as sex changes may influence broodstock management and larval production (Kamphausen et al., 2011; Zapata-Restrepo et al., 2019).

Environmental and management implications for ecosystem restoration and hatchery production

Our study provides new insights into the temporal and environmental regulation of *O. edulis* reproduction, offering practical guidance for conservation management and ecosystem restoration. By documenting the complete female spawning sequence, including ovulation, trans-ctenidial ovulation (TCO), fertilization, incubation, and larval release, we demonstrate that fertilization can occur under natural field conditions through rapid and tightly synchronized male-female spawning. Such fine-scale temporal coordination may help maintain reproductive success even when local effective densities are limited, although the potential contribution of surrounding populations cannot be excluded. Within this coordinated framework, however, residual temporal variability among individuals, including differences in spawning timing relative to astronomical cycles, as well as the presence of consistently earlier or later spawners, may represent a bet-hedging strategy that spreads reproductive risk and enhances recruitment stability under fluctuating environmental conditions. These findings improve our understanding of the species' reproductive ecology and offers a valuable basis for designing effective restoration strategies and ensuring a sustainable supply of high-quality seed oysters through breeding (e.g., hatchery; breeding-ponds) programs.

Environmental cycles, including annual, diel, lunar, tidal cues, strongly influence the reproductive timing. Understanding these relationships is essential for optimizing hatchery protocols, including the timing of spawning induction, broodstock management, timing of swarming, and larval rearing, as well as for informing restoration practices or *O. edulis* aquaculture practitioners, including the timing of settlement-substrate deployment (cultch) to maximize larval settlement while limiting premature colonization by competing organisms (Chapman et al., 2021) and promoting the development of a thin biofilm to induce settlement (Rodriguez-Perez et al., 2019). Future research should aim to identify the precise endogenous and environmental triggers for male and female spawning, evaluate synchronization across populations of different habitats, and detail the physiological mechanisms underlying TCO and incubation. Integrating behavioral, physiological, and molecular approaches will enhance our capacity to predict

reproductive success, improve hatchery breeding efficiency, and ensure the ecological viability of oyster populations.

While this study provides unique and novel *in situ* insights into the reproductive behavior of *O. edulis*, it is based on the continuous monitoring of a relatively limited number of individuals (n = 15). Nevertheless, continuous monitoring over 540 days generated an extensive dataset of behavioral observations, providing detailed insights into reproductive activity over time. Technological developments in long-term autonomous sensing systems are likely to soon enable monitoring larger numbers of individuals over extended temporal and geographical scales. Such approaches will open new perspectives for investigating population-level synchronization, spatial structuring of reproduction events, and potential chemical communication within natural oyster reef habitats in conserved and/or restored populations.

Conclusion

This study provides a detailed behavioral characterization of valve activity associated with reproduction events in *O. edulis*. By documenting, for the first time *in situ*, male spawning behavior, the complete sequence of female reproductive processes including ovulation, TCO, internal fertilization, incubation, and larval swarming, as well as the synchronization of these events with environmental cycles, the temperature-dependent modulation of incubation duration, and the occurrence and duration of sex changes, we confirm long-standing hypotheses, resolve historical ambiguities, and provide a substantial knowledge gain with new insights into the reproductive ecology of this endangered species and key ecosystem engineer. These findings carry direct implications for aquaculture and restoration, clarifying spawning triggers, incubation characteristics, and the role of sexual plasticity in population resilience. More broadly, they demonstrate how remote behavioral monitoring can transform the study of cryptic life-history processes in marine invertebrates, opening new perspectives on reproductive ecology and the vulnerability of reproductive synchrony under environmental change.

Aknowledgments

A. Le Moal was supported by a grant awarded to B. Meyer and B. Pogoda via AWI Inspires - International Science Program for Integrative Research in Earth Systems. The study contributes to the Helmholtz Research Program “Changing Earth-Sustaining our

future" of the research field Earth and Environment of the Helmholtz Association, Topic 6, Subtopic 6.1. and 6.2 as well as Topic 4, Suptopic 4.2. The project was supported by the "PHC PROCOPE" program via the project "OysterClock"(project-ID: 57701739), funded by the French Ministry for Europe and Foreign Affairs, the French Ministry for Higher Education and Research and the German Academic Exchange Service (DAAD). In addition, it was also supported by the French National Research Agency (ANR), LUCIOLE project (ANR-22-CE34- 0010-01).
We thank Tristan Hugh-Jones from ROSSMORE Oysters Ltd., for the oysters and his assistance with transport logistics to Helgoland. We thank the RESTORE and PROCEED projects teams, the AWI Helgoland Oyster Hatchery and the AWI Helgoland Oyster Nursery for their support with oyster cleaning, disinfection, acclimation and experimental setup, particularly Nicolás Araujo Piñeiro, Martin Sackmann, Henning Hoffmann, Nick Mackay-Roberts and Lennard Klingforth. Special thanks to Celina Behrendt for her help during oyster disinfection and during the design and the construction of the lander frames. We thank the AWI Scientific Workshop for designing and building the underwater lander for the experiment. We thank the AWI Centre for Scientific Diving (CSD) for their essential work during deployment and monthly sampling at Margate, especially Philipp Fischer, Markus Brand, Laura Eickelmann and Miriam Lienkämper, and all other divers involved. We also thank Anna Bergmann and Uwe Nettelmann for their assistance in general logistical support in the Helgoland laboratory. Finally, we thank Kristin Tessmar-Raible, Hélène Cochet, Lukas Hüppe and Dominik Bahlburg for their valuable discussions throughout the project.

Data availability

The datasets used and analysed during the current study (individual hourly valve behavior data (VOA) and temperature recordings) are publicly available in PANGAEA® - Data Publisher for Earth & Environmental Science(Le Moal et al., 2025).

References

Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., & Camus, L. (2016). High Frequency Non-invasive (HFNI) Bio-Sensors As a Potential Tool for Marine Monitoring and Assessments. *Frontiers in Marine Science*, *3*. https://doi.org/10.3389/fmars.2016.00187

Ayalon, I., Rosenberg, Y., Benichou, J. I. C., Campos, C. L. D., Sayco, S. L. G., Nada, M. A. L., Baquiran, J. I. P., Ligson, C. A., Avisar, D., Conaco, C., Kuechly, H. U., Kyba, C. C. M., Cabaitan, P. C., & Levy, O. (2021). Coral

Gametogenesis Collapse under Artificial Light Pollution. *Current Biology: CB*, *31*(2), 413-419.e3. https://doi.org/10.1016/j.cub.2020.10.039

Bayne, B. L. (2017). *Biology of Oysters*. Academic Press.

Bernard, I., Massabuau, J.-C., Ciret, P., Sow, M., Sottolichio, A., Pouvreau, S., & Tran, D. (2016). In situ spawning in a marine broadcast spawner, the Pacific oyster Crassostrea gigas: Timing and environmental triggers. *Limnology and Oceanography*, *61*(2), 635–647. https://doi.org/10.1002/lno.10240

Bernay, B., Baudy-Floc'h, M., Zanuttini, B., Zatylny-Gaudin, C., Pouvreau, S., & Henry, J. (2006). Ovarian and sperm regulatory peptides regulate ovulation in the oyster Crassostrea gigas. *Molecular Reproduction and Development*, *73*, 607–616. https://doi.org/10.1002/mrd.20472

Bloch, G., Barnes, B. M., Gerkema, M. P., & Helm, B. (2013). Animal activity around the clock with no overt circadian rhythms: Patterns, mechanisms and adaptive value. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20130019. https://doi.org/10.1098/rspb.2013.0019

Chaparro, O. R., Mardones-Toledo, D. A., Gray, M. W., Cubillos, V. M., Navarro, J. M., & Salas-Yanquin, L. P. (2018). Female-embryo relationships in Ostrea chilensis: Brooding, embryo recognition, and larval hatching. *Marine Biology*, *166*(1), 10. https://doi.org/10.1007/s00227-018-3457-1

Chaparro, O. R., Thompson, R. J., & Ward, J. E. (1993). In vivo Observations of Larval Brooding in the Chilean Oyster, Ostrea chilensis Philippi, 1845. *Biological Bulletin*, *185*(3), 365–372. https://doi.org/10.2307/1542477

Chapman, E. C. N., Rodriguez-Perez, A., Hugh-Jones, T., Bromley, C., James, M. A., Diele, K., & Sanderson, W. G. (2021). Optimising recruitment in habitat creation for the native European oyster (*Ostrea edulis*): Implications of temporal and spatial variability in larval abundance. *Marine Pollution Bulletin*, *170*, 112579. https://doi.org/10.1016/j.marpolbul.2021.112579

Cole, H. A. (1942). Primary Sex-Phases in Ostrea edulis. *Journal of Cell Science*, *s2-83*(331), 317–356. https://doi.org/10.1242/jcs.s2-83.331.317

Colsoul, B., Boudry, P., Pérez-Parallé, M. L., Bratoš Cetinić, A., Hugh-Jones, T., Arzul, I., Mérou, N., Wegner, K. M., Peter, C., Merk, V., & Pogoda, B. (2021). Sustainable large-scale production of European flat oyster (Ostrea edulis) seed for ecological restoration and aquaculture: A review. *Reviews in Aquaculture*, *13*(3), 1423–1468. https://doi.org/10.1111/raq.12529

Culloty, S. C., & Mulcahy, M. F. (2007). *Bonamia ostreae in the Native Oyster Ostrea edulis*. https://oar.marine.ie/handle/10793/269

Davies, T. W., Levy, O., Tidau, S., de Barros Marangoni, L. F., Wiedenmann, J., D'Angelo, C., & Smyth, T. (2023). Global disruption of coral broadcast spawning associated with artificial light at night. *Nature Communications*, *14*(1), 2511. https://doi.org/10.1038/s41467-023-38070-y

Dutertre, M., Beninger, P. G., Barillé, L., Papin, M., Rosa, P., Barillé, A.-L., & Haure, J. (2009). Temperature and seston quantity and quality effects on field reproduction of farmed oysters, Crassostrea gigas, in Bourgneuf Bay, France. *Aquatic Living Resources*, *22*(3), 319–329. https://doi.org/10.1051/alr/2009042

Edwards, M., & Richardson, A. J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, *430*(7002), 881–884. https://doi.org/10.1038/nature02808

Erdmann, W. (1935). *Uber die Entwicklung und die Anatomie der" ansatzreifen" Larve von Ostrea edulis*. *19 (1)*, 25.

Fabioux, C., Huvet, A., Le Souchu, P., Le Pennec, M., & Pouvreau, S. (2005). Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. *Aquaculture*, *250*(1), 458–470. https://doi.org/10.1016/j.aquaculture.2005.02.038

Fischer, P., Brix, H., Baschek, B., Kraberg, A., Brand, M., Cisewski, B., Riethmüller, R., Breitbach, G., Möller, K. O., Gattuso, J.-P., Alliouane, S., van de Poll, W. H., & Witbaard, R. (2020). Operating Cabled Underwater Observatories in Rough Shelf-Sea Environments: A Technological Challenge. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00551

Fournier, J., Levesque, E., Pouvreau, S., Pennec, M. L., & Moullac, G. L. (2012). Influence of plankton concentration on gametogenesis and spawning of the black lip pearl oyster *Pinctada margaritifera* in Ahe atoll lagoon (Tuamotu archipelago, French polynesia). *Marine Pollution Bulletin, Ahe Atoll and Pearl Oyster Aquaculture in the Tuamotu Archipelago*, *65*(10), 463–470. https://doi.org/10.1016/j.marpolbul.2012.03.027

Galtsoff, P. S. (1938a). Physiology of reproduction of ostrea virginica: I. spawning reactions of the female and male. *The Biological Bulletin*, *74*(3), 461–486. https://doi.org/10.2307/1537816

Galtsoff, P. S. (1938b). Physiology of reproduction of ostrea virginica: Ii. stimulation of spawning in the female oyster. *The Biological Bulletin*, *75*(2), 286–307. https://doi.org/10.2307/1537736

Gray, M., Salas-Yanquin, L., Büchner-Miranda, J., & Chaparro, O. (2022). The Ostrea chilensis pallial cavity: Nursery, prison, and time machine. *Marine Biology*, *169*. https://doi.org/10.1007/s00227-021-04015-6

Hamel, J.-F., & Mercier, A. (1996). Evidence of Chemical Communication During the Gametogenesis of Holothuroids. *Ecology*, *77*(5), 1600–1616. https://doi.org/10.2307/2265555

Harrison, P. L., Babcock, R. C., Bull, G. D., Oliver, J. K., Wallace, C. C., & Willis, B. L. (1984). Mass Spawning in Tropical Reef Corals. *Science*, *223*(4641), 1186–1189. https://doi.org/10.1126/science.223.4641.1186

Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., & Bloch, G. (2017). Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1734), 20160246. https://doi.org/10.1098/rstb.2016.0246

Henshaw, J. M., Marshall, D. J., Jennions, M. D., & Kokko, H. (2014). Local Gamete Competition Explains Sex Allocation and Fertilization Strategies in the Sea. *The American Naturalist*, *184*(2), E32–E49. https://doi.org/10.1086/676641

His, E., Beiras, R., & Seaman, M. N. L. (1999). *The Assessment of Marine Pollution—Bioassays with Bivalve Embryos and Larvae*. *37*, 1–178. https://doi.org/10.1016/S0065-2881(08)60428-9

Kamphausen, L., Jensen, A., & Hawkins, L. (2011). Unusually High Proportion of Males in a Collapsing Population of Commercially Fished Oysters (Ostrea edulis) in the Solent, United Kingdom. *Journal of Shellfish Research*, *30*(2), 217–222. https://doi.org/10.2983/035.030.0204

Korringa, P. (1940). *Experiments End Observations on Swarming, Pelagic Life and Setting in the European Flat Oyster, Ostrea Edulis L.: Contribution from the Governmental Institution for Biological Fishery Research*. s.e.

Korringa, P. (1947). Relations between the Moon and Periodicity in the Breeding of Marine Animals. *Ecological Monographs*, *17*(3), 347–381. https://doi.org/10.2307/1948665

Korringa, P. (1952). Recent Advances in Oyster Biology. *The Quarterly Review of Biology*, *27*(3), 266–308. https://doi.org/10.1086/399023

Le Moal, A., Payton, L., Andrade, H., Camus, L., Ballantine, C., Ciret, P., & Tran, D. (2023). Blue mussels' valve behavior exhibits daily and lunar rhythms during the high Arctic polar day. *Marine Biology*, *170*(9), 113. https://doi.org/10.1007/s00227-023-04257-6

Le Moal, A., Payton, L., Geerebaert, Y., Meyer, B., Pogoda, B., & Tran, D. (2025). *Valve behavior of the European flat oyster Ostrea edulis and associated underwater sound and temperature data, recorded during a 18-month in situ experiment in Helgoland, Germany* [Dataset bundled publication]. PANGAEA. https://doi.pangaea.de/10.1594/PANGAEA.983652

Le Moal, A., Tran, D., Payton, L., Geerebaert, Y., Pogoda, B., & Meyer, B. (2026). The phenology of the European flat oyster (Ostrea edulis) and its environmental drivers. *Scientific Reports*, *16*(1), 23359. https://doi.org/10.1038/s41598-026-63105-x

Levitan, D. R., Fogarty, N. D., Jara, J., Lotterhos, K. E., & Knowlton, N. (2011). Genetic, spatial, and temporal components of precise spawning synchrony in reef-building corals of the Montastraea annularis species complex. *Evolution*, *65*(5), 1254–1270. https://doi.org/10.1111/j.1558-5646.2011.01235.x

Levitan, D. R., & Petersen, C. (1995). Sperm limitation in the sea. *Trends in Ecology & Evolution*, *10*(6), 228–231. https://doi.org/10.1016/S0169-5347(00)89071-0

Lubet, p. (1991). Reproduction des mollusques [Reproduction of molluscs]. In *Bases Biologiques et Ecologiques de l'Aquaculture* (pp. 168–204).

Maneiro, V., Silva, A., Pazos, A. J., Sánchez, J. L., & Pérez-Parallé, M. L. (2017). Effects of temperature and photoperiod on the conditioning of the flat oyster (Ostrea edulis L.) in autumn. *Aquaculture Research*, *48*(8), 4554–4562. https://doi.org/10.1111/are.13280

Mardones-Toledo, D. A., Montory, J. A., Joyce, A., Thompson, R. J., Diederich, C. M., Pechenik, J. A., Mardones, M. L., & Chaparro, O. R. (2015). Brooding in the Chilean Oyster Ostrea chilensis: Unexpected Complexity in the Movements of Brooded Offspring within the Mantle Cavity. *PLOS ONE*, *10*(4), e0122859. https://doi.org/10.1371/journal.pone.0122859

Marteil, L. (1976). La conchyliculture francaise. 2eme partie. Biologie de l'huitre et de la moule. *Revue Des Travaux de l'Institut Des Pêches Maritimes*, *40*(2), 149–346.

McClintock, J. B., & Watts, S. A. (1990). The effects of photoperiod on gametogenesis in the tropical sea urchin *Eucidaris tribuloides* (Lamarck) (Echinodermata: Echinoidea). *Journal of Experimental Marine Biology and Ecology*, *139*(3), 175–184. https://doi.org/10.1016/0022-0981(90)90145-3

Mercier, A., & Hamel, J.-F. (2010). Synchronized breeding events in sympatric marine invertebrates: Role of behavior and fine temporal windows in maintaining reproductive isolation. *Behavioral Ecology and Sociobiology*, *64*(11), 1749–1765. https://doi.org/10.1007/s00265-010-0987-z

Nelson, T. C. (1928). Relation of Spawning of the Oyster to Temperature. *Ecology*, *9*(2), 145–154. https://doi.org/10.2307/1929351

Ó Foighil, D., & Taylor, D. J. (2000). Evolution of Parental Care and Ovulation Behavior in Oysters. *Molecular Phylogenetics and Evolution*, *15*(2), 301–313. https://doi.org/10.1006/mpev.1999.0755

Orton, J. H. (1927). Observation and Experiments on Sex-Change in the European Oyster (O. edulis.): Part I. The Change from Female to Male. *Journal of the Marine Biological Association of the United Kingdom*, *14*(4), 967–1045. https://doi.org/10.1017/S0025315400051183

OSPAR. (2009). *Background document for Ostrea edulis and Ostrea edulis beds*. In Biodiversity Series 428. Edited by Jan Haelters and Francis Kerckhof. Paris: OSPAR Commission, 22p.

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(Volume 37, 2006), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Payton, L., Sow, M., Massabuau, J.-C., Ciret, P., & Tran, D. (2017). How annual course of photoperiod shapes seasonal behavior of diploid and triploid oysters, Crassostrea gigas. *PLOS ONE*, *12*(10), e0185918. https://doi.org/10.1371/journal.pone.0185918

Pogoda, B. (2019). Current Status of European Oyster Decline and Restoration in Germany. *Humanities*, *8*(1), Article 1. https://doi.org/10.3390/h8010009

Pogoda, B., Boudry, P., Bromley, C., Cameron, T. C., Colsoul, B., Donnan, D., Hancock, B., Hugh-Jones, T., Preston, J., Sanderson, W. G., Sas, H., Brown, J., Bonacic, K., von Nordheim, H., & zu Ermgassen, P. S. E. (2020). NORA moving forward: Developing an oyster restoration network in Europe to support the Berlin Oyster Recommendation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2031–2037. https://doi.org/10.1002/aqc.3447

Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., Sanderson, W., & Nordheim, H. von. (2019). The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation: Bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquatic Living Resources*, *32*, 13. https://doi.org/10.1051/alr/2019012

Pouvreau, S., Lapègue, S., Arzul, I., & Boudry, P. (2023). Fifty years of research to counter the decline of the European flat oyster (Ostrea edulis): A review of French achievements and prospects for the restoration of remaining beds and revival of aquaculture production. *Aquatic Living Resources*, *36*, 13. https://doi.org/10.1051/alr/2023006

Prytherch, H. F. (1929). *Investigation of the Physical Conditions Controlling Spawning of Oysters and the Occurrence, Distribution, and Setting of Oyster Larvae in Milford Harbor, Connecticut*. U.S. Government Printing Office.

R Core Team. (2023). *R: A language and environment for statistical computing*. [Computer software]. Foundation for Statistical Computing. URL https://www.R-project.org/

Rato, A., Joaquim, S., Matias, D., & Hubbard, P. C. (2025). The Roles of Chemical Cues in the Life Cycle of Bivalves: Spawning, Settlement, and Metamorphosis. *Reviews in Aquaculture*, *17*(1), e13007. https://doi.org/10.1111/raq.13007

Rice, P., Ray, S. M., Painter, S. J., & Nagle, G. T. (2002). An intrinsic membrane protein in oyster sperm stimulates spawning behaviors in Crassostrea virginica: Implications for aquaculture. *Journal of Shellfish Research*, *21*, 715–718.

Rodriguez-Perez, A., James, M., Donnan, D. W., Henry, T. B., Møller, L. F., & Sanderson, W. G. (2019). *Conservation and restoration of a keystone species: Understanding the settlement preferences of the European oyster (Ostrea edulis)*. https://doi.org/10.1016/j.marpolbul.2018.11.032

Sáenz de Miera, C., Monecke, S., Bartzen-Sprauer, J., Laran-Chich, M.-P., Pévet, P., Hazlerigg, D. G., & Simonneaux, V. (2014). A Circannual Clock Drives Expression of Genes Central for Seasonal Reproduction. *Current Biology*, *24*(13), 1500–1506. https://doi.org/10.1016/j.cub.2014.05.024

Sas, H., Deden, B., Kamermans, P., Ermgassen, P. S. E. zu, Pogoda, B., Preston, J., Helmer, L., Holbrook, Z., Arzul, I., Have, T. van der, Villalba, A., Colsoul, B., Lown, A., Merk, V., Zwerschke, N., & Reuchlin, E. (2020). Bonamia infection in native oysters (Ostrea edulis) in relation to European restoration projects. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2150–2162. https://doi.org/10.1002/aqc.3430

Slattery, M., Hines, G. A., Starmer, J., & Paul, V. J. (1999). Chemical signals in gametogenesis, spawning, and larval settlement and defense of the soft coral Sinularia polydactyla. *Coral Reefs*, *18*(1), 75–84. https://doi.org/10.1007/s003380050158

Spärck, R. (1925). Studies on the biology of the oyster (Ostrea edulis) in the Limfjord with special reference to the influence of temperature on the sex change. *In Commission by GEC Gad.*

Starr, M., Himmelman, J. H., & Therriault, J. C. (1990). Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science (New York, N.Y.)*, *247*(4946), 1071–1074. https://doi.org/10.1126/science.247.4946.1071

Suquet, M., Pouvreau, S., Queau, I., Boulais, M., Grand, J. L., Ratiskol, D., & Cosson, J. (2018). Biological characteristics of sperm in European flat oyster (Ostrea edulis). *Aquatic Living Resources*, *31*, 20. https://doi.org/10.1051/alr/2018008

Sweeney, A., Boch, C., Johnsen, S., & Morse, D. (2011). Twilight spectral dynamics and the coral reef invertebrate spawning response. *The Journal of Experimental Biology*, *214*, 770–777. https://doi.org/10.1242/jeb.043406

Thurstan, R. H., McCormick, H., Preston, J., Ashton, E. C., Bennema, F. P., Cetinić, A. B., Brown, J. H., Cameron, T. C., Costa, F. da, Donnan, D., Ewers, C., Fortibuoni, T., Galimany, E., Giovanardi, O., Grancher, R., Grech, D., Hayden-Hughes, M., Helmer, L., Jensen, K. T., … Ermgassen, P. S. zu. (2023). *Historical dataset details the distribution, extent and form of lost Ostrea edulis reef ecosystems.* https://ecoevorxiv.org/repository/view/6359/

Tonk, L., Witbaard, R., Dalen, P. van, Cheng, C. H., & Kamermans, P. (2023). Applicability of the gape monitor to study flat oyster (Ostrea edulis) feeding behaviour. *Aquatic Living Resources*, *36*(6), 6. https://doi.org/10.1051/alr/2022021

Tran, D., Andrade, H., Camus, L., Leopold, P., Ballantine, C., Berge, J., Durier, G., Sow, M., & Ciret, P. (2023). Evidence of separate influence of moon and sun on light synchronization of mussel's daily rhythm during the polar night. *iScience*, *26*(3). https://doi.org/10.1016/j.isci.2023.106168

Visser, M. E., & Gienapp, P. (2019). Evolutionary and demographic consequences of phenological mismatches. *Nature Ecology & Evolution*, *3*(6), 879–885. https://doi.org/10.1038/s41559-019-0880-8

Walne, P. (1974). *Culture of Bivalve Molluscs: 50 Years Experience at Conwy*. Fishing News Books Limited, Surrey.

Waltham, N. J., Elliott, M., Lee, S. Y., Lovelock, C., Duarte, C. M., Buelow, C., Simenstad, C., Nagelkerken, I., Claassens, L., Wen, C. K.-C., Barletta, M., Connolly, R. M., Gillies, C., Mitsch, W. J., Ogburn, M. B., Purandare, J., Possingham, H., & Sheaves, M. (2020). UN Decade on Ecosystem Restoration 2021–2030—What Chance for Success in Restoring Coastal Ecosystems? *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00071

Yerushalmi, S., & Green, R. M. (2009). Evidence for the adaptive significance of circadian rhythms. *Ecology Letters*, *12*(9), 970–981. https://doi.org/10.1111/j.1461-0248.2009.01343.x

Yonge, C. M. (1960). *Oysters*. Collins.

Yund, P. O. (2000). How severe is sperm limitation in natural populations of marine free-spawners? *Trends in Ecology & Evolution*, *15*(1), 10–13. https://doi.org/10.1016/S0169-5347(99)01744-9

Zapata-Restrepo, L. M., Hauton, C., Williams, I. D., Jensen, A. C., & Hudson, M. D. (2019). Effects of the interaction between temperature and steroid hormones on gametogenesis and sex ratio in the European flat oyster (*Ostrea edulis*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *236*, 110523. https://doi.org/10.1016/j.cbpa.2019.06.023

zu Ermgassen, P., Hancock, B., B, D., Greene, J., E, S., Spalding, M., & R., B. (2016). *Setting Objectives for Oyster Habitat Restoration Using Ecosystem Services: A Manager's Guide.*

zu Ermgassen, P., Thurstan, R. H., Corrales, J., Alleway, H., Carranza, A., Dankers, N., DeAngelis, B., Hancock, B., Kent, F., McLeod, I., Pogoda, B., Liu, Q., & Sanderson, W. G. (2020). The benefits of bivalve reef restoration: A global synthesis of underrepresented species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2050–

7. Discussion and perspectives

Prior to this work, little was known about the circadian and seasonal organization of the European flat oyster, *O. edulis*, representing a critical gap in both basic and applied ecology. This thesis represents the first comprehensive chronobiological investigation of *O. edulis*. By integrating laboratory and *in situ* behavioral, growth, reproductive, and molecular data, it provides new insights into the temporal organization of this keystone bivalve species. Historically widespread across European coastal ecosystems, *O. edulis* has experienced dramatic declines due to overfishing, habitat destruction, disease, and invasive species (Pouvreau et al., 2023; Thurstan et al., 2023; zu Ermgassen et al., 2025). Restoration initiatives seek to reverse these losses, yet their success requires a mechanistic understanding of how oysters functioning in dynamic environments. Chronobiology, which examines how organisms have evolved internal clocks to synchronize with environmental cycles, provides a powerful framework for evaluating the resilience and ecological integration of restored populations (Helm et al., 2017; Monecke, 2024). Our results permitted us to address three key questions regarding the chronobiology of *O. edulis*: (i) does this species possess a functional circadian clock, detectable at both behavioral and molecular levels, (ii) how are behavioral and growth rhythms expressed in the field and shaped by environmental drivers, and (iii) how do temporal mechanisms coordinate reproduction in this species?

7.1 Advances in the understanding of chronobiology of *Ostrea edulis*

7.1.1 Behavioral rhythms and endogenous control

A central objective of this thesis was to determine whether O. edulis possesses a functional circadian clock detectable at the behavioral level. Laboratory experiments confirmed that diel rhythms of valve opening persisted under constant dark conditions, providing clear evidence of an internal circadian clock (chapter 3 and 4). This intrinsic timekeeping mechanism shows that *O. edulis*, like other bivalves (Garcia-March et al., 2016; Mat et al., 2012; Tran, Andrade, et al., 2020; Tran et al., 2023), does not simply respond passively to environmental fluctuations but is able to anticipate them. Such

predictive ability is central in chronobiology, as it allows organisms to prepare for cyclic changes in their environment rather than passively responding (Pittendrigh, 1960). The detection of circadian rhythmicity under controlled conditions provides an essential framework for interpreting field results (chapter 3 and 4). *In situ*, daily rhythms in valve activity were consistently observed, but because environmental cues are always present, their endogenous origin cannot be directly proven. However, these rhythms showed strong seasonal modulation, with a higher proportion of oysters exhibiting rhythmicity in spring and summer, and fewer rhythmic individuals in autumn and winter (chapter 5). By linking the laboratory evidence of circadian control with these field observations, we infer that the diel rhythms observed *in situ* are at least partly driven by an endogenous clock, while their expression is modulated by seasonal environmental variability. This integration of circadian and circannual processes may optimize energy allocation by synchronizing activity with resource availability and environmental constraints (Gwinner, 2012; Helm et al., 2017; Visser et al., 2010).

Field data further revealed the coexistence of multiple rhythmic components in valve activity of *O. edulis*. Patterns consistent with tidal, semilunar, and lunar periodicities were observed, although their strength and detectability varied seasonally (chapter 5). For example, tidal rhythms were evident during spring and summer but totally absent in fall and winter. This seasonal disappearance most likely reflects the general winter slowdown in activity and metabolism, which dampens the expression of rhythmic behaviors. The detection of semilunar and lunar rhythms may be linked to both mechanical forcing and photic detection of moonlight via photoreceptor proteins (Andreatta & Tessmar-Raible, 2020; Payton & Tran, 2019). In O. edulis, daily rhythms are robust under light:dark conditions in the laboratory, but free-running experiments reveal labile oscillations at the individual level, allowing rapid resynchronization to environmental shifts, a capacity confirmed during the field experiment (chapter 3 and 5). Together, these results establish at the behavioral level that O. edulis possesses a functional endogenous circadian clock. Recognizing this endogenous control is essential for understanding how oysters allocate energy and maintain synchrony in variable natural conditions. However, while such rhythms were observed, caution is warranted in interpreting them as evidence of separate endogenous oscillators. Our work demonstrates

circadian control in the laboratory, but the existence of distinct circatidal or circalunar clocks remains speculative and requires targeted experimentation.

7.1.2 Molecular clockwork identification and temporal gene expression

Another key objective of this thesis was to determine at the molecular level whether *O. edulis* possesses a functional circadian clock. To address this, I first researched and identified 13 putative clock genes in *O. edulis*, including orthologs of core clock and clock-associated genes (*OeClock*, *OeBmal1*, *OePeriod*, *OeTimeless*, *OeCryptochrome2*, *OeRev-erb*, *OeRor*, *OeDoubletime*, *OeClockWorkOrange*, *OeShaggy*), a gene related to melatonin synthesis (*OeHiomt*), and genes involved in light perception (*OeCryptochrome1*, *OeOpsin4*) (chapter 4). The identification of these genes, together with the characterization of key protein domains, their phylogenetic classification, and the analysis of their relative expression, allowed us to deduce their putative roles in O. edulis. This extends prior work in C. gigas and confirms that bivalves share a conserved, flexible circadian system (Perrigault & Tran, 2017). In particular, the detection of two distinct cryptochromes supports a "butterfly-type" organization, as previously described in the butterfly Danaus plexippus, the copepod Calanus finmarchicus, and C. gigas (Christie et al., 2013; Perrigault & Tran, 2017; Zhu et al., 2005). Such duplication may underlie functional diversification, with *OeCryptochrome1* acting as a light-sensitive photoreceptor and *OeCryptochrome2* as a transcriptional repressor in the transcription-translation feedback loop.

Expression profiling further confirmed that these genes are not only present but also rhythmically regulated, providing functional evidence of circadian machinery in *O. edulis*. Rhythms were expressed at circadian, ultradian, and annual scales depending on the gene and tissue (chapter 4 and 5). Core clock genes, including OeClock, OeBmal1, OePeriod, and OeTimeless, showed tissue-specific rhythmicity, with some genes exhibiting daily oscillations and others ultradian patterns depending on the tissue. This variability is consistent with their role in maintaining endogenous rhythmicity while allowing plastic adjustment to local physiological demands (chapter 4). As an interesting

example, we detected annual rhythmicity in OeHiomt, encoding hydroxyindole-O-methyltransferase, an enzyme involved in melatonin synthesis (Foulkes et al., 1997). Melatonin is known to play a role in both circadian and seasonal regulation in vertebrates (Reiter, 1993; Tosches et al., 2014), suggesting that putative clock-controlled components like OeHiomt may also contribute to seasonal timing in oysters, although functional validation is still required. Another notable gene, OeOpn4, a putative photoreceptor, also showed rhythmic expression, highlighting the potential role of light-mediated regulation in coordinating both daily and seasonal physiological processes in *O. edulis* (chapter 4 and 5).

Together, these molecular results provide the first evidence that *O. edulis* could possess a functional endogenous circadian clock at the molecular level. The identification of rhythmic expression in canonical clock genes demonstrates that circadian machinery is active under both laboratory and field conditions, providing a mechanistic basis for the behavioral rhythms documented in this thesis (chapter 3, 4 and 5). While our data do not yet establish circatidal or circalunar oscillators, they strongly suggest that the circadian molecular system interacts with environmental cues to structure behavior and physiology. Recognizing this endogenous molecular control is crucial, as it links gene regulation with observed daily, seasonal, and reproductive cycles, and offers a framework for assessing physiological synchrony in restoration contexts.

7.2 Advances in the understanding of *Ostrea edulis* behavior and response to environmental parameters

Having established that *O. edulis* possesses a functional circadian clock at the laboratory level, the next question concerns how these rhythms are expressed in the field and shaped by environmental drivers. Our *in situ* experiments revealed that behavior and growth in *O. edulis* are strongly rhythmic, structured by diel, tidal, semilunar, lunar, and seasonal cycles rather than occurring randomly (chapter 5). Valve activity, a central integrative measure of bivalve physiology, consistently showed daily rhythmicity, while shell growth also aligned with predictable cycles (chapter 5). Among these drivers, seasonal variability emerged as the dominant factor: activity and growth peaked in spring and summer,

whereas winter was characterized by reduced metabolism and activity, resembling a quiescence state (chapter 5). This seasonal slowdown mirrors strategies observed in crustaceans such as copepods (Baumgartner & Tarrant, 2017) or in the mussel *Mytilus spp.* in the Arctic (Le Moal et al., 2023; Tran, Andrade, et al., 2020), which suppress activity during periods of cold and food scarcity. Such convergence suggests a widespread adaptation among ectotherms to balance energetic costs with seasonal resource availability (Gwinner, 2012; Visser et al., 2010). Photoperiod and temperature were identified as the primary cues orchestrating valve behavior (chapter 5). Photoperiod, as a highly reliable signal of seasonal progression, acts as the principal *zeitgeber* for circannual rhythms, indirectly shaping other factors such as temperature and food availability (Gwinner, 1989; Helm & Lincoln, 2017; Visser et al., 2010). Temperature, by contrast, plays a modulatory role, directly influencing metabolic rates. These findings are consistent with previous studies in *C. gigas* and other bivalves, which demonstrated photoperiod-dependent seasonal behavior in reproduction and growth (Fabioux et al., 2005; Payton, Sow, et al., 2017). Additional environmental factors, including chlorophyll *a*, salinity, turbidity, and underwater sound variation, acted as modulators of behavior and growth. The ability of *O. edulis* to integrate multiple cues highlights its ecological plasticity, enabling adaptation to complex estuarine and coastal environments where multiple cycles overlap and interact.

From an adaptive perspective, this plasticity allows *O. edulis* to synchronize energy use, growth, and reproduction with dynamic environments, but may also create vulnerabilities to anthropogenic perturbations. Artificial Light At Night (ALAN) is a growing concern in coastal ecosystems, as it can disrupt photoperiodic entrainment (Davies et al., 2014; Gaston et al., 2017). Studies in *C. gigas* have shown that ALAN can affects circadian rhythmicity and lead to severe physiological and ecological consequences (Botté, Payton, Lefeuvre, et al., 2023; Botté, Payton, & Tran, 2023a, 2023b), preliminary results in *O. edulis* suggest similar susceptibility (chapter 3). Quantifying the effects of ALAN on molecular, behavioral, and reproductive rhythms will be critical to establish thresholds for effective management. Climate change poses additional challenges, as warming oceans may decouple photoperiodic cues from temperature signals, potentially generating phenological mismatches in feeding, growth, and reproduction (Bradshaw & Holzapfel, 2010; Helm et al., 2013). Anthropogenic noise

and altered turbidity or salinity represent additional stressors: sound can act both as a *zeitgeber* and a stressor altering valve activity (Charifi et al., 2017; Peng et al., 2015; Tidau & Briffa, 2016), while turbidity and salinity fluctuations disrupt feeding efficiency, reproductive success, osmoregulation and impact fitness (Pourmozaffar et al., 2020; Shumway, 1977; Tuttle-Raycraft & Ackerman, 2019).

This work therefore contributes to filling a knowledge gap in the ecology of *O. edulis*. These findings show that *O. edulis* behavior in the field is structured by overlapping geophysical cycles, with seasonal modulation as a dominant feature. Recognizing this organization is essential not only for understanding oyster ecology but also for restoration efforts. Until now, restoration strategies have emphasized disease resistance, habitat suitability, and genetic diversity, while neglecting the temporal dimension. By demonstrating how valve activity, growth, and reproduction are synchronized with environmental cycles, this work establishes chronobiology as a missing but essential axis for evaluating resilience and ecological integration in reintroduced populations.

7.3 Contribution to the understanding of *Ostrea edulis* reproduction

Another major contribution of this thesis is the continuous, *in situ* description of *O. edulis* reproductive behavior using high-frequency valvometry (chapter 6). This work revealed a highly stereotyped sequence of valve behaviors during male spawning and female spawning (ovulation, trans-ctenidial ovulation, incubation, and larval swarming). These detailed patterns, previously only described in older studies or observed in hatchery conditions (Bayne, 2017; Galtsoff, 1938a), provide new insight into reproductive coordination and timing. Unlike the well-studied *C. gigas*, *O. edulis* is a larviparous, protandric hermaphrodite that performs internal fertilization (Mardones-Toledo et al., 2015), and our approach allowed a more precise characterization of its reproductive behavior in natural conditions (chapter 6).

Reproductive behaviors in *O. edulis* were synchronized both with environmental cycles and between males and females (chapter 6). Male spawning consistently preceded female spawning by minutes, ensuring sperm availability at the time of egg release and suggesting, as indicated in previous studies (Nelson & Allison, 1940; Suquet et al., 2018), that male spawning could act as a trigger for the onset of female ovulation. These

behaviors were also strongly aligned with lunar cycles, with spawning peaking during full moon phases and larval release occurring preferentially around new moon phases, consistent with earlier observations by (Korringa, 1947). Incubation duration varied with temperature, reflecting thermal plasticity in larval development as documented in other oysters and bivalves (Bayne, 2017; Colsoul et al., 2021). Together, these results provide mechanistic insight into how environmental cues and endogenous timing interact to coordinate reproduction, optimizing fertilization success and larval survival, a critical consideration for population recovery and restoration efforts.

Our observations of rapid, consecutive hermaphroditism also have significant implications. In several individuals, we detected rapid sex changes within a single breeding season, with cases of two successive female incubations occurring during the same summer (chapter 6). This reveals a higher degree of reproductive flexibility than previously documented (Bayne, 2017; Yonge, 1960). By enabling sex inversion on such a short timescale, *O. edulis* minimizes the risk of skewed sex ratios, a particularly important mechanism in small or recovering populations where fertilization success may be limited. This reproductive plasticity not only highlights an adaptive strategy that likely contributed to the historical ecological success of the species but also represents a valuable trait for restoration and reintroduction, where maintaining effective reproductive output is essential for population recovery. By documenting the full sequence of reproductive behaviors in the field, this work fills a fundamental knowledge gap in the ecology of *O. edulis*.

7.4 Integrative perspectives: linking behavior, molecular clocks, and reproduction

Taken together, the four studies presented in this thesis reveal a hierarchical but flexible temporal organization in *O. edulis* (Fig. 7.1). At the molecular level, a conserved circadian machinery structures daily rhythm (chapter 3 and 4). At the behavioral level, valve activity and growth integrate circadian and seasonal signals, further modulated by environmental cycles (chapter 5). At the reproductive level, spawning and larval release are synchronized with lunar and seasonal windows that optimize reproductive success

(chapter 6). Endogenous clocks allow oysters to anticipate environmental cycles, while entrainment mechanisms ensure fine-tuning to local variability. This multi-scale organization suggests that *O. edulis* relies on both internal oscillators and external cues to align its life history with predictable geophysical rhythms (Fig. 7.1).

Our findings also highlight evolutionary adaptation. The presence of an endogenous circadian clock, confirmed under laboratory conditions, shows that oysters maintain rhythmic organization even in the absence of external signals (chapter 3 and 4). Field observations then demonstrate how these rhythms are modulated and synchronized by environmental cycles, reflecting co-evolutionary adaptation to coastal variability (chapter 5). Such alignment likely contributes to fitness by optimizing feeding, growth, and reproduction under fluctuating conditions (Fig. 7.1). At the same time, it offers guidance for restoration: if reintroduced populations retain their ability to resynchronize with natural cycles, sustainable reestablishment is possible, provided anthropogenic disruptions are minimized.

These integrative insights extend beyond *O. edulis*. They illustrate the importance of treating biological time as a key dimension of marine ecology and restoration. Temporal organization is not an accessory trait but a central axis of adaptation, shaping how organisms interact with ecosystems and respond to environmental change (Häfker et al., 2023; Helm et al., 2017; Mermet et al., 2017). By bridging molecular, behavioral, and ecological levels, this thesis contributes to building a framework for marine chronobiology, with particular relevance for *O. edulis* reef recovery.

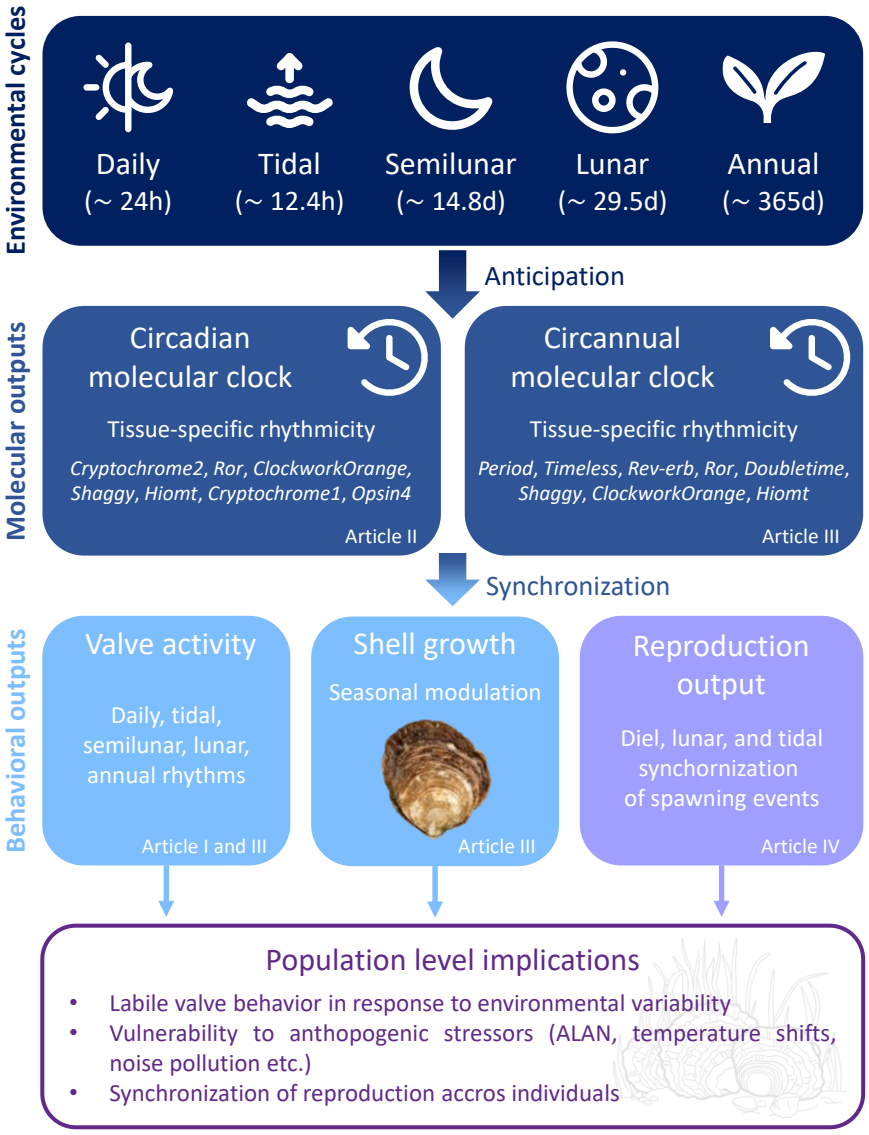


Figure 7.1. Conceptual framework of multi-scale rhythms in the European flat oyster (**O. edulis**). Arrows indicate hierarchical connections between the different levels.

7.5 Conclusion and perspectives for restoration and reintroduction projects

This thesis establishes the first integrative chronobiological framework for *O. edulis*, linking molecular, behavioral, growth, and reproductive processes. Our results permitted us to address three key questions regarding the chronobiology of *O. edulis*: (i) whether this species possesses a functional circadian clock detectable at both behavioral and molecular levels, (ii) how behavioral and growth rhythms are expressed in the field and

shaped by environmental drivers, and (iii) how temporal mechanisms coordinate reproduction. Its main contributions are: (i) demonstration of an endogenous circadian clock regulating valve activity (chapter 3), (ii) molecular characterization of circadian components, including the identification of canonical clock genes and the observation of tissue-specific circadian, ultradian, and annual rhythmicity in some genes (chapter 4), (iii) identification of seasonal modulation and multi-scale behavioral rhythms aligned with geophysical cycles (chapter 5), and (iv) the first *in situ* description of the full reproductive sequence, from spawning to larval release and sex change (chapter 6).

Together, these findings demonstrate that *O. edulis* rhythms arise from an interplay between endogenous clocks and environmental cycles, providing adaptive advantages in dynamic coastal environments but also exposing vulnerabilities under anthropogenic pressures. Beyond advancing fundamental chronobiology, they highlight practical applications for restoration, where aligning interventions with natural rhythms may enhance reintroduction success.

Looking ahead, the key challenge is to better resolve the molecular basis of circannual timing, the interactions between multiple oscillators, and the resilience of oysters under long-term environmental change. These questions form the foundation for the perspectives that follow, where chronobiology and restoration ecology converge to guide the recovery of *O. edulis* in European coastal ecosystems.

7.5.1 Implications for restoration and reintroduction

Understanding the chronobiology of *O. edulis* offers direct applications for restoration and reintroduction programs. Effective site selection should account not only for habitat suitability but also for the integrity of environmental cues that regulate circadian and seasonal rhythms. Exposure to artificial light at night, altered tidal regimes, acoustic disturbances, or other anthropogenic stressors may compromise clock entrainment, affecting feeding behavior, growth, and reproductive synchrony, and ultimately reducing population fitness. Integrating knowledge of these potential disruptions into planning allows managers to anticipate and mitigate their impacts.

Timing of restoration interventions is another critical consideration. Larval release and seeding efforts aligned with optimal seasonal and lunar windows can maximize

recruitment success, ensuring that reintroduced populations synchronize with natural environmental cycles. Molecular and behavioral chronobiological markers can serve as bioindicators of population health and rhythmic coherence, providing practical tools to monitor the effectiveness of restoration efforts and detect early signs of stress or maladaptation.

Population-specific rhythmic plasticity, particularly along latitudinal gradients, should also be considered when selecting broodstock. Differences in spawning behavior, reproductive timing, and environmental response among populations suggest that local adaptation may influence the success of reintroduction under varying environmental conditions. By combining these insights, restoration strategies can enhance both the resilience and ecological integration of restored populations. Chronobiology thus adds a temporal dimension to restoration, emphasizing that successful reestablishment is not solely about numbers or habitat availability, but about reestablishing alignment with the natural rhythms of coastal ecosystems.

7.5.2 Open questions and future directions

Despite these advances, significant knowledge gaps remain. The molecular mechanisms of circannual timing are unresolved, though suggested by seasonal oscillations in gene expression and behavior (chapter 5). Inter-population variability is another frontier. Oysters across latitudinal gradients may exhibit differences in rhythmic plasticity, reproductive synchrony, and environmental sensitivity (Bayne, 2017; Colsoul et al., 2021). Understanding such variability is essential for selecting appropriate broodstock for restoration. Additionally, the long-term impacts of chronic anthropogenic disruption remain poorly understood. Multi-generational studies are needed to determine whether plasticity confers resilience or whether populations gradually lose synchrony under sustained stress. Finally, future work should also examine potential chemical communication and social synchronization within reef populations, processes that may reinforce collective rhythms in these gregarious bivalves. Addressing these questions will not only clarify the chronobiology of *O. edulis* but will also contribute to broader marine chronobiology, linking molecular mechanisms with population dynamics and ecosystem processes.

8. References cited in Introduction and Discussion

Abhilash, L., Shindey, R., & Sharma, V. K. (2017). To be or not to be rhythmic? A review of studies on organisms inhabiting constant environments. *Biological Rhythm Research*, *48*(5), 677–691. https://doi.org/10.1080/09291016.2017.1345426

Adema, C. M. (2021). Sticky problems: Extraction of nucleic acids from molluscs. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1825), 20200162. https://doi.org/10.1098/rstb.2020.0162

Airoldi, L., Balata, D., & Beck, M. W. (2008). The Gray Zone: Relationships between habitat loss and marine diversity and their applications in conservation. *Journal of Experimental Marine Biology and Ecology*, *366*(1), 8–15. https://doi.org/10.1016/j.jembe.2008.07.034

Airoldi, L., & Beck, M. W. (2007). Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology*, *45*, 345–405.

Ameyaw-Akumfi, C., & Naylor, E. (1987). Temporal patterns of shell-gape in Mytilus edulis. *Marine Biology*, *95*(2), 237–242. https://doi.org/10.1007/BF00409011

Andrade, H., Massabuau, J.-C., Cochrane, S., Ciret, P., Tran, D., Sow, M., & Camus, L. (2016). High Frequency Non-invasive (HFNI) Bio-Sensors As a Potential Tool for Marine Monitoring and Assessments. *Frontiers in Marine Science*, *3*. https://doi.org/10.3389/fmars.2016.00187

Andreatta, G., & Tessmar-Raible, K. (2020). The Still Dark Side of the Moon: Molecular Mechanisms of Lunar-Controlled Rhythms and Clocks. *Journal of Molecular Biology*, *432*(12), 3525–3546. https://doi.org/10.1016/j.jmb.2020.03.009

Arboleda, E., Zurl, M., Waldherr, M., & Tessmar-Raible, K. (2019). Differential Impacts of the Head on Platynereis dumerilii Peripheral Circadian Rhythms. *Frontiers in Physiology*, *10*, 900. https://doi.org/10.3389/fphys.2019.00900

Aschoff, J. (1955). Jahresperiodik der Fortpflanzung bei Warmblütern. *Studium Generale*, *8*(12), 742–776.

Aschoff, J. (1958). Tierische Periodik unter dem Einfluß von Zeitgebern. *Zeitschrift Für Tierpsychologie*, *15*(1), 1–30. https://doi.org/10.1111/j.1439-0310.1958.tb00552.x

Aschoff, J. (1960). Exogenous and Endogenous Components in Circadian Rhythms. *Cold Spring Harbor Symposia on Quantitative Biology*, *25*, 11–28. https://doi.org/10.1101/SQB.1960.025.01.004

Aschoff, J. (1981). Freerunning and Entrained Circadian Rhythms. In J. Aschoff (Ed.), *Biological Rhythms* (pp. 81–93). Springer US. https://doi.org/10.1007/978-1-4615-6552-9_6

Atkinson, A., Hill, S. L., Pakhomov, E. A., Siegel, V., Reiss, C. S., Loeb, V. J., Steinberg, D. K., Schmidt, K., Tarling, G. A., Gerrish, L., & Sailley, S. F. (2019). Krill (Euphausia superba) distribution contracts southward during rapid regional warming. *Nature Climate Change*, *9*(2), 142–147. https://doi.org/10.1038/s41558-018-0370-z

Ayalon, I., Rosenberg, Y., Benichou, J. I. C., Campos, C. L. D., Sayco, S. L. G., Nada, M. A. L., Baquiran, J. I. P., Ligson, C. A., Avisar, D., Conaco, C., Kuechly, H. U., Kyba, C. C. M., Cabaitan, P. C., & Levy, O. (2021). Coral Gametogenesis Collapse under Artificial Light Pollution. *Current Biology: CB*, *31*(2), 413-419.e3. https://doi.org/10.1016/j.cub.2020.10.039

Baghel, K., & Pati, A. (2015). Pheromones as time cues for circadian rhythms in fish. *Biological Rhythm Research*, *46*, 1–18. https://doi.org/10.1080/09291016.2015.1046246

Bamber, S. D. (2023). Valve gaping behaviour in the European oyster (*Ostrea edulis*) in response to changes in light intensity when combined with variations in salinity and seawater temperature. *Journal of Experimental Marine Biology and Ecology*, *568*, 151943. https://doi.org/10.1016/j.jembe.2023.151943

Bargiello, T. A., & Young, M. W. (1984). Molecular genetics of a biological clock in Drosophila. *Proceedings of the National Academy of Sciences*, *81*(7), 2142–2146. https://doi.org/10.1073/pnas.81.7.2142

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Baumgartner, M. F., & Tarrant, A. M. (2017). The Physiology and Ecology of Diapause in Marine Copepods. *Annual Review of Marine Science*, *9*(Volume 9, 2017), 387–411. https://doi.org/10.1146/annurev-marine-010816-060505

Bayne, B. L. (1969). The gregarious behaviour of the larvae of Ostrea edulis L. at settlement. *Journal of the Marine Biological Association of the United Kingdom*, *49*(2), 327–356. https://doi.org/10.1017/S0025315400035943

Bayne, B. L. (2017). *Biology of Oysters*. Academic Press.

Beaugrand, G., Conversi, A., Atkinson, A., Cloern, J., Chiba, S., Fonda-Umani, S., Kirby, R. R., Greene, C. H., Goberville, E., Otto, S. A., Reid, P. C., Stemmann, L., & Edwards, M. (2019). Prediction of unprecedented biological shifts in the global ocean. *Nature Climate Change*, *9*(3), 237–243. https://doi.org/10.1038/s41558-019-0420-1

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., Defeo, O., Edgar, G. J., Hancock, B., Kay, M. C., Lenihan, H. S., Luckenbach, M. W., Toropova, C. L., Zhang, G., & Guo, X. (2011). Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *BioScience*, *61*(2), 107–116. https://doi.org/10.1525/bio.2011.61.2.5

Bellingham, J., Whitmore, D., Philp, A. R., Wells, D. J., & Foster, R. G. (2002). Zebrafish melanopsin: Isolation, tissue localisation and phylogenetic position. *Molecular Brain Research*, *107*(2), 128–136. https://doi.org/10.1016/S0169-328X(02)00454-0

Bell-Pedersen, D., Cassone, V. M., Earnest, D. J., Golden, S. S., Hardin, P. E., Thomas, T. L., & Zoran, M. J. (2005). Circadian rhythms from multiple oscillators: Lessons from diverse organisms. *Nature Reviews Genetics*, *6*(7), 544–556. https://doi.org/10.1038/nrg1633

Beninger, P. G., St-Jean, S. D., & Poussart, Y. (1995). Labial palps of the blue mussel Mytilus edulis (Bivalvia: Mytilidae). *Marine Biology*, *123*(2), 293–303. https://doi.org/10.1007/BF00353621

Bennema, F. P., Engelhard, G. H., & Lindeboom, H. (2020). Ostrea edulis beds in the central North Sea: Delineation, ecology, and restoration. *ICES Journal of Marine Science*, *77*(7–8), 2694–2705. https://doi.org/10.1093/icesjms/fsaa134

Berghahn, R., & Ruth, M. (2005). The disappearance of oysters from the Wadden Sea: A cautionary tale for no-take zones. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*(1), 91–104. https://doi.org/10.1002/aqc.635

Bernard, I., Massabuau, J.-C., Ciret, P., Sow, M., Sottolichio, A., Pouvreau, S., & Tran, D. (2016). In situ spawning in a marine broadcast spawner, the Pacific oyster Crassostrea gigas: Timing and environmental triggers. *Limnology and Oceanography*, *61*(2), 635–647. https://doi.org/10.1002/lno.10240

Bernay, B., Baudy-Floc'h, M., Zanuttini, B., Zatylny-Gaudin, C., Pouvreau, S., & Henry, J. (2006). Ovarian and sperm regulatory peptides regulate ovulation in the oyster Crassostrea gigas. *Molecular Reproduction and Development*, *73*, 607–616. https://doi.org/10.1002/mrd.20472

Bertolini, E., Schubert, F. K., Zanini, D., Sehadová, H., Helfrich-Förster, C., & Menegazzi, P. (2019). Life at High Latitudes Does Not Require Circadian Behavioral Rhythmicity under Constant Darkness. *Current Biology*, *29*(22), 3928-3936.e3. https://doi.org/10.1016/j.cub.2019.09.032

BfN. (2020a). Managementplan für das Naturschutzgebiet "Borkum Riffgrund." *MPBRg Bundesamt Für Naturschutz, Bonn (Eds.)*.

BfN. (2020b). Managementplan für das Naturschutzgebiet "Sylter Außenriff—Östliche Deutsche Bucht." *Bundesamt Für Naturschutz, Bonn (Eds.)*.

Bingham, C., Arbogast, B., Guillaume, G. C., Lee, J. K., & Halberg, F. (1982). Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia*, *9*(4), 397–439.

Biscontin, A., Wallach, T., Sales, G., Grudziecki, A., Janke, L., Sartori, E., Bertolucci, C., Mazzotta, G., De Pittà, C., Meyer, B., Kramer, A., & Costa, R. (2017). Functional characterization of the circadian clock in the Antarctic krill, Euphausia superba. *Scientific Reports*, *7*(1), 17742. https://doi.org/10.1038/s41598-017-18009-2

Bloch, G., Barnes, B. M., Gerkema, M. P., & Helm, B. (2013). Animal activity around the clock with no overt circadian rhythms: Patterns, mechanisms and adaptive value. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20130019. https://doi.org/10.1098/rspb.2013.0019

Bloch, G., Bar-Shai, N., Cytter, Y., & Green, R. (2017). Time is honey: Circadian clocks of bees and flowers and how their interactions may influence ecological communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1734), 20160256. https://doi.org/10.1098/rstb.2016.0256

Boissin, J., & Canguilhem, B. (1998). *Les rythmes du vivant: Origine et contrôle des rythmes biologiques*. Nathan: CNRS éd.

Bonham, V., & Roberts, D. (2018). Ostrea edulis (European oyster). *CABI Compendium*, *CABI Compendium*, 71177. https://doi.org/10.1079/cabicompendium.71177

Boos, K., Buchholz, C., Buchholz, F., & Gutow, L. (2004). *Bericht über die Zusammensetzung des Helgoländer Makrozoobenthos im Vergleich historischer und aktueller Quellen—Klassifizierungsvorschlag nach der WRRL und Empfehlungen zum Monitoring*. https://agris.fao.org/search/en/providers/122415/records/6473687308fd68d54605e8c3

Botté, A., Payton, L., Lefeuvre, E., & Tran, D. (2023). Is part-night lighting a suitable mitigation strategy to limit Artificial Light at Night effects on the biological rhythm at the behavioral and molecular scales of the oyster *Crassostrea gigas*? *Science of The Total Environment*, *905*, 167052. https://doi.org/10.1016/j.scitotenv.2023.167052

Botté, A., Payton, L., & Tran, D. (2023a). Artificial light at night at environmental intensities disrupts daily rhythm of the oyster *Crassostrea gigas*. *Marine Pollution Bulletin*, *191*, 114850. https://doi.org/10.1016/j.marpolbul.2023.114850

Botté, A., Payton, L., & Tran, D. (2023b). The effects of artificial light at night on behavioral rhythm and related gene expression are wavelength dependent in the oyster Crassostrea gigas. *Environmental Science and Pollution Research*, *30*(57), 120375–120386. https://doi.org/10.1007/s11356-023-30793-1

Box, G. E. P., Jenkins, G. M., & Reinsel, G. C. (1994). *Time series analysis: Forecasting and control* (Third edition). Prentice Hall. http://www.gbv.de/dms/bowker/toc/9780130607744.pdf

Bradshaw, W. E., & Holzapfel, C. M. (2010). Light, Time, and the Physiology of Biotic Response to Rapid Climate Change in Animals. *Annual Review of Physiology*, *72*(Volume 72, 2010), 147–166. https://doi.org/10.1146/annurev-physiol-021909-135837

Brierley, A. S., & Kingsford, M. J. (2009). Impacts of Climate Change on Marine Organisms and Ecosystems. *Current Biology*, *19*(14), R602–R614. https://doi.org/10.1016/j.cub.2009.05.046

Bruce, J. R., Knight, M., & Parke, M. W. (1940). The rearing of oyster larvae on an algal diet. *Journal of the Marine Biological Association of the United Kingdom*, *24*(1), 337–374. https://doi.org/10.1017/S0025315400054606

Buestel, D., Ropert, M., Prou, J., Goulletquer, P., Prou, J., & Goulletquer, P. (2009). History, Status, and Future of Oyster Culture in France. *Journal of Shellfish Research*, *28*(4), 813–820. https://doi.org/10.2983/035.028.0410

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Campbell, G. (2012). *Oysters ancient and modern: Potential shape variation with habitat in flat oysters (Ostrea edulis L.), and its possible use in archaeology*. https://www.academia.edu/1560528/Oysters_ancient_and_modern_potential_shape_variation_with_habitat_in_flat_oysters_Ostrea_edulis_L_and_its_possible_use_in_archaeology

Caspers, H. (1950). Die Lebensgemeinschaft der Helgoländer Austernbank. *Helgoländer wissenschaftliche Meeresuntersuchungen*, *3*(1), 119–169. https://doi.org/10.1007/BF02252090

Cermakian, N., & Sassone-Corsi, P. (2000). Multilevel regulation of the circadian clock. *Nature Reviews Molecular Cell Biology*, *1*(1), 59–67. https://doi.org/10.1038/35036078

Chaparro, O. R., Mardones-Toledo, D. A., Gray, M. W., Cubillos, V. M., Navarro, J. M., & Salas-Yanquin, L. P. (2018). Female–embryo relationships in Ostrea chilensis: Brooding, embryo recognition, and larval hatching. *Marine Biology*, *166*(1), 10. https://doi.org/10.1007/s00227-018-3457-1

Chapman, E. C., O'Dell, A. R., Meligi, N. M., Parsons, D. R., & Rotchell, J. M. (2017). Seasonal expression patterns of clock-associated genes in the blue mussel Mytilus edulis. *Chronobiology International*, *34*(9), 1300–1314. https://doi.org/10.1080/07420528.2017.1363224

Charifi, M., Sow, M., Ciret, P., Benomar, S., & Massabuau, J.-C. (2017). The sense of hearing in the Pacific oyster, Magallana gigas. *PLOS ONE*, *12*(10), e0185353. https://doi.org/10.1371/journal.pone.0185353

Chaves, I., Pokorny, R., Byrdin, M., Hoang, N., Ritz, T., Brettel, K., Essen, L.-O., Horst, G. T. J. van der, Batschauer, A., & Ahmad, M. (2011). The Cryptochromes: Blue Light Photoreceptors in Plants and Animals. *Annual Review of Plant Biology*, *62*(Volume 62, 2011), 335–364. https://doi.org/10.1146/annurev-arplant-042110-103759

Christie, A. E., Fontanilla, T. M., Nesbit, K. T., & Lenz, P. H. (2013). Prediction of the protein components of a putative *Calanus finmarchicus* (Crustacea, Copepoda) circadian signaling system using a *de novo* assembled transcriptome. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *8*(3), 165–193. https://doi.org/10.1016/j.cbd.2013.04.002

Cole, H. A. (1937). Metamorphosis of the Larva of Ostrea edulis. *Nature*, *139*(3514), 413–414. https://doi.org/10.1038/139413b0

Cole, H. A. (1938). The Fate of the Larval Organs in the Metamorphosis of Ostrea Edulis. *Journal of the Marine Biological Association of the United Kingdom*, *22*(2), 469–484. https://doi.org/10.1017/S0025315400012376

Cole, H. A. (1941). The fecundity of Ostrea edulis. *Journal of the Marine Biological Association of the United Kingdom*, *25*(2), 243–260. https://doi.org/10.1017/S0025315400054710

Cole, H. A. (1942). Primary Sex-Phases in Ostrea edulis. *Journal of Cell Science*, *s2-83*(331), 317–356. https://doi.org/10.1242/jcs.s2-83.331.317

Colsoul, B., Boudry, P., Pérez-Parallé, M. L., Bratoš Cetinić, A., Hugh-Jones, T., Arzul, I., Mérou, N., Wegner, K. M., Peter, C., Merk, V., & Pogoda, B. (2021). Sustainable large-scale production of European flat oyster (Ostrea edulis) seed for ecological restoration and aquaculture: A review. *Reviews in Aquaculture*, *13*(3), 1423–1468. https://doi.org/10.1111/raq.12529

Council of the European Union. (1992). *Council Directive 92/43/EEC of 21 May 1992 on the Conservation of Natural Habitats and of wild Fauna and Flora*. Brussels: European Parliament.

Council of the European Union. (2008). Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 Establishing a Framework for Community Action in the Field of Marine Environmental Policy (Marine Strategy Framework Directive). *Brussels: European Parliament*, 19–40.

Council of the European Union. (2024). Regulation (EU) 2024/1991 of the European Parliament and of the Council of 24 June 2024 on nature restoration and amending Regulation (EU) 2022/869. *Brussels: European Parliament*.

Culloty, S. C., & Mulcahy, M. F. (2007). *Bonamia ostreae in the Native Oyster Ostrea edulis*. https://oar.marine.ie/handle/10793/269

Cyran, S. A., Buchsbaum, A. M., Reddy, K. L., Lin, M.-C., Glossop, N. R. J., Hardin, P. E., Young, M. W., Storti, R. V., & Blau, J. (2003). *Vrille*, *Pdp1*, and *dClock* Form a Second Feedback Loop in the *Drosophila* Circadian Clock. *Cell*, *112*(3), 329–341. https://doi.org/10.1016/S0092-8674(03)00074-6

Daan, S. (2010). A History of Chronobiological Concepts. In U. Albrecht (Ed.), *The Circadian Clock* (pp. 1–35). Springer. https://doi.org/10.1007/978-1-4419-1262-6_1

Dardente, H., Dardente, H., & Cermakian, N. (2007). Molecular Circadian Rhythms in Central and Peripheral Clocks in Mammals. *Chronobiology International*, *24*(2), 195–213. https://doi.org/10.1080/07420520701283693

Davies, T. W., Bennie, J., Cruse, D., Blumgart, D., Inger, R., & Gaston, K. J. (2017). Multiple night-time light-emitting diode lighting strategies impact grassland invertebrate assemblages. *Global Change Biology*, *23*(7), 2641–2648. https://doi.org/10.1111/gcb.13615

Davies, T. W., Duffy, J. P., Bennie, J., & Gaston, K. J. (2014). The nature, extent, and ecological implications of marine light pollution. *Frontiers in Ecology and the Environment*, *12*(6), 347–355. https://doi.org/10.1890/130281

Davies, T. W., Duffy, J. P., Bennie, J., & Gaston, K. J. (2016). Stemming the Tide of Light Pollution Encroaching into Marine Protected Areas. *Conservation Letters*, *9*(3), 164–171. https://doi.org/10.1111/conl.12191

Davies, T. W., & Smyth, T. (2018). Why artificial light at night should be a focus for global change research in the 21st century. *Global Change Biology*, *24*(3), 872–882. https://doi.org/10.1111/gcb.13927

Davison, A., & Neiman, M. (2021). Mobilizing molluscan models and genomes in biology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *376*(1825), 20200163. https://doi.org/10.1098/rstb.2020.0163

De Bruyne, R., van Leeuwen, S., Gmelig Meyling, A., & Daan, R. (2013). *Schelpdieren van het Nederlandse Noordzeegebied: Ecologische atlas van de mariene weekdieren (Mollusca)*. https://www.waterbouwkundiglaboratorium.be/nl/open-wl-archief

De Mairan, J. (1729). Observation botanique. *Histoire de l'Academie Royale Des Sciences Paris*. https://cir.nii.ac.jp/crid/1573105975426983936

Denlinger, D. L. (Ed.). (2022). Interpreting Seasonal Cues to Program Diapause Entry. In *Insect Diapause* (pp. 57–120). Cambridge University Press. https://doi.org/10.1017/9781108609364.006

Dodd, A. N., Salathia, N., Hall, A., Kévei, E., Tóth, R., Nagy, F., Hibberd, J. M., Millar, A. J., & Webb, A. A. R. (2005). Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and Competitive Advantage. *Science*, *309*(5734), 630–633. https://doi.org/10.1126/science.1115581

Du, Y., Zhang, L., Xu, F., Huang, B., Zhang, G., & Li, L. (2013). Validation of housekeeping genes as internal controls for studying gene expression during Pacific oyster (*Crassostrea gigas*) development by quantitative real-time PCR. *Fish & Shellfish Immunology*, *34*(3), 939–945. https://doi.org/10.1016/j.fsi.2012.12.007

Dubruille, R., & Emery, P. (2008). A Plastic Clock: How Circadian Rhythms Respond to Environmental Cues in Drosophila. *Molecular Neurobiology*, *38*(2), 129–145. https://doi.org/10.1007/s12035-008-8035-y

Dunlap, J. C. (1999). Molecular Bases for Circadian Clocks. *Cell*, *96*(2), 271–290. https://doi.org/10.1016/S0092-8674(00)80566-8

Dunlap, J. C., Loros, J. J., & DeCoursey, P. J. (2004). *Chronobiology: Biological timekeeping* (pp. xix, 406). Sinauer Associates.

Emerson, K. J., Bradshaw, W. E., & Holzapfel, C. M. (2008). Concordance of the circadian clock with the environment is necessary to maximize fitness in natural populations. *Evolution; International Journal of Organic Evolution*, *62*(4), 979–983. https://doi.org/10.1111/j.1558-5646.2008.00324.x

Emery, P., So, W. V., Kaneko, M., Hall, J. C., & Rosbash, M. (1998). CRY, a Drosophila Clock and Light-Regulated Cryptochrome, Is a Major Contributor to Circadian Rhythm Resetting and Photosensitivity. *Cell*, *95*(5), 669–679. https://doi.org/10.1016/S0092-8674(00)81637-2

Emery, P., Stanewsky, R., Helfrich-Förster, C., Emery-Le, M., Hall, J. C., & Rosbash, M. (2000). Drosophila CRY Is a Deep Brain Circadian Photoreceptor. *Neuron*, *26*(2), 493–504. https://doi.org/10.1016/S0896-6273(00)81181-2

Enright, J. T. (1976). Plasticity in an isopod's clockworks: Shaking shapes form and affects phase and frequency. *Journal of Comparative Physiology*, *107*(1), 13–37. https://doi.org/10.1007/BF00663916

Erdmann, W. (1935). *Uber die Entwicklung und die Anatomie der" ansatzreifen" Larve von Ostrea edulis*. *19 (1)*, 25.

Fabioux, C., Huvet, A., Le Souchu, P., Le Pennec, M., & Pouvreau, S. (2005). Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. *Aquaculture*, *250*(1), 458–470. https://doi.org/10.1016/j.aquaculture.2005.02.038

Fabra, M., Morrall, Z., Helmer, L., Watson, G., & Preston, J. (2024). Filtration Behaviour of Ostrea edulis: Diurnal Rhythmicity Influenced by Light Cycles, Body Size and Water Temperature. *Estuaries and Coasts*, *48*(1), 18. https://doi.org/10.1007/s12237-024-01453-5

Falchi, F., Cinzano, P., Duriscoe, D., Kyba, C. C. M., Elvidge, C. D., Baugh, K., Portnov, B. A., Rybnikova, N. A., & Furgoni, R. (2016). The new world atlas of artificial night sky brightness. *Science Advances*, *2*(6), e1600377. https://doi.org/10.1126/sciadv.1600377

FAO. (2024). *Fishery and Aquaculture Statistics – Yearbook 2021*. FAO ; https://openknowledge.fao.org/handle/20.500.14283/cc9523en

Fischer, P., Brix, H., Baschek, B., Kraberg, A., Brand, M., Cisewski, B., Riethmüller, R., Breitbach, G., Möller, K. O., Gattuso, J.-P., Alliouane, S., van de Poll, W. H., & Witbaard, R. (2020). Operating Cabled Underwater Observatories in Rough Shelf-Sea Environments: A Technological Challenge. *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00551

Fischer, P., Lienkämper, M., Happel, L., Bussmann, I., Eickelmann, L., Anselm, N., Brand, M., & Brix, H. (2024). *Hydrographical time series data of Helgoland, Southern North Sea, 2023* [Dataset]. PANGAEA. https://doi.org/10.1594/PANGAEA.967855

Foulkes, N. S., assone-Corsi, P., Borjigin, J., & Snyder, S. H. (1997). Rhythmic transcription: The molecular basis of circadian melatonin synthesis. *Trends in Neurosciences*, *20*(10), 487–492. https://doi.org/10.1016/S0166-2236(97)01109-0

Fournier, J., Levesque, E., Pouvreau, S., Pennec, M. L., & Moullac, G. L. (2012). Influence of plankton concentration on gametogenesis and spawning of the black lip pearl oyster *Pinctada margaritifera* in Ahe atoll lagoon (Tuamotu archipelago, French polynesia). *Marine Pollution Bulletin*, *65*(10), 463–470. https://doi.org/10.1016/j.marpolbul.2012.03.027

Fox, J., & Weisberg, S. (2018). An R companion to applied regression. *Sage Publications*.

Galler, S., Litzlbauer, J., Kröss, M., & Grassberger, H. (2009). The highly efficient holding function of the mollusc 'catch' muscle is not based on decelerated myosin head cross-bridge cycles. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1682), 803–808. https://doi.org/10.1098/rspb.2009.1618

Galtsoff, P. S. (1938a). Physiology of reproduction of ostrea virginica: I. spawning reactions of the female and male. *The Biological Bulletin*, *74*(3), 461–486. https://doi.org/10.2307/1537816

Galtsoff, P. S. (1938b). Physiology of reproduction of ostrea virginica: Ii. stimulation of spawning in the female oyster. *The Biological Bulletin*, *75*(2), 286–307. https://doi.org/10.2307/1537736

Garcia-March, J. R., Jiménez, S., Sanchis, M. A., Monleon, S., Lees, J., Surge, D., & Tena-Medialdea, J. (2016). In situ biomonitoring shows seasonal patterns and environmentally mediated gaping activity in the bivalve, Pinna nobilis. *Marine Biology*, *163*(2), 29. https://doi.org/10.1007/s00227-016-2812-3

García-March, J. R., Sanchís Solsona, M. Á., & García-Carrascosa, A. M. (2008). Shell gaping behaviour of Pinna nobilis L., 1758: Circadian and circalunar rhythms revealed by in situ monitoring. *Marine Biology*, *153*(4), 689–698. https://doi.org/10.1007/s00227-007-0842-6

Gaston, K. J., Davies, T. W., Nedelec, S. L., & Holt, L. A. (2017). Impacts of Artificial Light at Night on Biological Timings. *Annual Review of Ecology, Evolution, and Systematics*, *48*(Volume 48, 2017), 49–68. https://doi.org/10.1146/annurev-ecolsys-110316-022745

Gercken, J., & Schmidt, A. (2014). Current status of the European Oyster (Ostrea edulis) and possibilities for restoration in the German North Sea. *Bundesamt Für Naturschutz*.

Glossop, N. R., Lyons, L. C., & Hardin, P. E. (1999). Interlocked feedback loops within the Drosophila circadian oscillator. *Science (New York, N.Y.)*, *286*(5440), 766–768. https://doi.org/10.1126/science.286.5440.766

Gosling, E. (2003). *Bivalve Molluscs: Biology, Ecology and Culture*. John Wiley & Sons.

Goto, S. G. (2013). Roles of circadian clock genes in insect photoperiodism. *Entomological Science*, *16*(1), 1–16. https://doi.org/10.1111/ens.12000

Gray, M., Salas-Yanquin, L., Büchner-Miranda, J., & Chaparro, O. (2022). The Ostrea chilensis pallial cavity: Nursery, prison, and time machine. *Marine Biology*, *169*. https://doi.org/10.1007/s00227-021-04015-6

Guillaumond, F., Dardente, H., Giguère, V., & Cermakian, N. (2005). Differential Control of Bmal1 Circadian Transcription by REV-ERB and ROR nuclear Receptors. *Journal of Biological Rhythms*, *20*(5), 391–403. https://doi.org/10.1177/0748730405277232

Gundappa, M. K., Peñaloza, C., Regan, T., Boutet, I., Tanguy, A., Houston, R. D., Bean, T. P., & Macqueen, D. J. (2022). Chromosome-level reference genome for European flat oyster (Ostrea edulis L.). *Evolutionary Applications*, *15*(11), 1713–1729. https://doi.org/10.1111/eva.13460

Guo, H., Brewer, J. M., Lehman, M. N., & Bittman, E. L. (2006). Suprachiasmatic regulation of circadian rhythms of gene expression in hamster peripheral organs: Effects of transplanting the pacemaker. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *26*(24), 6406–6412. https://doi.org/10.1523/JNEUROSCI.4676-05.2006

Gwinner, E. (1981). Circannual Systems. In J. Aschoff (Ed.), *Biological Rhythms* (pp. 391–410). Springer US. https://doi.org/10.1007/978-1-4615-6552-9_21

Gwinner, E. (1989). Photoperiod as a Modifying and Limiting Factor in the Expression of Avian Circannual Rhythms. *Journal of Biological Rhythms*, *4*(2), 125–138. https://doi.org/10.1177/074873048900400210

Gwinner, E. (2012). *Circannual Rhythms: Endogenous Annual Clocks in the Organization of Seasonal Processes*. Springer Science & Business Media.

Gwinner, E., & Brandstatter, R. (2001). Complex bird clocks. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *356*(1415), 1801–1810. https://doi.org/10.1098/rstb.2001.0959

Haelters, J., & Kerckhof, F. (2009). *Background document for Ostrea edulis and Ostrea edulis bed*. OSPAR Commission – Biodiversity Series, pp. 1–22. Anonymous, London.

Häfker, N. S., Andreatta, G., Manzotti, A., Falciatore, A., Raible, F., & Tessmar-Raible, K. (2023). Rhythms and Clocks in Marine Organisms. *Annual Review of Marine Science*, *15*(Volume 15, 2023), 509–538. https://doi.org/10.1146/annurev-marine-030422-113038

Häfker, N. S., Meyer, B., Last, K. S., Pond, D. W., Hüppe, L., & Teschke, M. (2017). Circadian Clock Involvement in Zooplankton Diel Vertical Migration. *Current Biology*, *27*(14), 2194-2201.e3. https://doi.org/10.1016/j.cub.2017.06.025

Häfker, N. S., Teschke, M., Last, K. S., Pond, D. W., Hüppe, L., & Meyer, B. (2018). Calanus finmarchicus seasonal cycle and diapause in relation to gene expression, physiology, and endogenous clocks. *Limnology and Oceanography*, *63*(6), 2815–2838. https://doi.org/10.1002/lno.11011

Hagmeier, A., & Kändler, r. (1927). Neue Untersuchungen im nordfriesischen Wattenmeer und auf den fiskalischen Austernbänken. *Lipsius & Tischer*.

Hankins, M. W., Peirson, S. N., & Foster, R. G. (2008). Melanopsin: An exciting photopigment. *Trends in Neurosciences*, *31*(1), 27–36. https://doi.org/10.1016/j.tins.2007.11.002

Hardin, P. E. (2005). The Circadian Timekeeping System of Drosophila. *Current Biology*, *15*(17), R714–R722. https://doi.org/10.1016/j.cub.2005.08.019

Hardin, P. E., Hall, J. C., & Rosbash, M. (1990). Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. *Nature*, *343*(6258), 536–540. https://doi.org/10.1038/343536a0

Harper, R. (2017). *"Time Flies" Multisensory Processing by Circadian Clocks in Drosophila melanogaster*.

Hastings, M. H. (1981). The entraining effect of turbulence on the circa-tidal activity rhythm and its semi-lunar modulation in Eurydice pulchra. *Journal of the Marine Biological Association of the United Kingdom*, *61*(1), 151–160. https://doi.org/10.1017/S0025315400045987

Helm, B., Ben-Shlomo, R., Sheriff, M. J., Hut, R. A., Foster, R., Barnes, B. M., & Dominoni, D. (2013). Annual rhythms that underlie phenology: Biological time-keeping meets environmental change. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20130016. https://doi.org/10.1098/rspb.2013.0016

Helm, B., & Lincoln, G. A. (2017). Circannual Rhythms Anticipate the Earth's Annual Periodicity. In V. Kumar (Ed.), *Biological Timekeeping: Clocks, Rhythms and Behaviour* (pp. 545–569). Springer India. https://doi.org/10.1007/978-81-322-3688-7_26

Helm, B., Visser, M. E., Schwartz, W., Kronfeld-Schor, N., Gerkema, M., Piersma, T., & Bloch, G. (2017). Two sides of a coin: Ecological and chronobiological perspectives of timing in the wild. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1734), 20160246. https://doi.org/10.1098/rstb.2016.0246

Hendricks, W. D., Byrum, C. A., & Meyer-Bernstein, E. L. (2012). Characterization of circadian behavior in the starlet sea anemone, Nematostella vectensis. *PloS One*, *7*(10), e46843. https://doi.org/10.1371/journal.pone.0046843

Henshaw, J. M., Marshall, D. J., Jennions, M. D., & Kokko, H. (2014). Local Gamete Competition Explains Sex Allocation and Fertilization Strategies in the Sea. *The American Naturalist*, *184*(2), E32–E49. https://doi.org/10.1086/676641

Herrero, M. J., & Lepesant, J. M. J. (2014). Daily and seasonal expression of clock genes in the pituitary of the European sea bass (*Dicentrarchus labrax*). *General and Comparative Endocrinology*, *208*, 30–38. https://doi.org/10.1016/j.ygcen.2014.08.002

His, E., Beiras, R., & Seaman, M. N. L. (1999). *The Assessment of Marine Pollution—Bioassays with Bivalve Embryos and Larvae*. *37*, 1–178. https://doi.org/10.1016/S0065-2881(08)60428-9

Hölker, F., Bolliger, J., Davies, T. W., Giavi, S., Jechow, A., Kalinkat, G., Longcore, T., Spoelstra, K., Tidau, S., Visser, M. E., & Knop, E. (2021). 11 Pressing Research Questions on How Light Pollution Affects Biodiversity. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.767177

Hunt, B. J. (2016). *Advancing molecular crustacean chronobiology through the characterisation of the circadian clock in two malacostracan species, Euphausia superba and Parhyale hawaiensis* [Thesis, University of Leicester]. https://figshare.le.ac.uk/articles/thesis/Advancing_molecular_crustacean_chronobiology_through_the_characterisation_of_the_circadian_clock_in_two_malacostracan_species_Euphausia_superba_and_Parhyale_hawaiensis/10209509/1

Hüppe, L., Payton, L., Last, K., Wilcockson, D., Ershova, E., & Meyer, B. (2020). Evidence for oscillating circadian clock genes in the copepod Calanus finmarchicus during the summer solstice in the high Arctic. *Biology Letters*, *16*(7), 20200257. https://doi.org/10.1098/rsbl.2020.0257

Ikegami, K., & Yoshimura, T. (2017). Molecular Mechanism Regulating Seasonality. In V. Kumar (Ed.), *Biological Timekeeping: Clocks, Rhythms and Behaviour* (pp. 589–605). Springer India. https://doi.org/10.1007/978-81-322-3688-7_28

Jamil, T., Opdekamp, W., van Diggelen, R., & ter Braak, C. J. F. (2012). Trait-Environment Relationships and Tiered Forward Model Selection in Linear Mixed Models. *International Journal of Ecology*, *2012*(1), 947103. https://doi.org/10.1155/2012/947103

Jamil, T., Ozinga, W. A., Kleyer, M., & ter Braak, C. J. F. (2013). Selecting traits that explain species–environment relationships: A generalized linear mixed model approach. *Journal of Vegetation Science*, *24*(6), 988–1000. https://doi.org/10.1111/j.1654-1103.2012.12036.x

Jetten, A. M., & Joo, J. H. (2006). Retinoid-related orphan receptors (RORs): Roles in cellular differentiation and development. In *Advances in Developmental Biology* (Vol. 16, pp. 313–355). Elsevier. https://doi.org/10.1016/S1574-3349(06)16010-X

Jodrey, L. H. (1953). Studies on Shell Formation. III. Measurement of Calcium Deposition in Shell and Calcium Turnover in Mantle Tissue using the Mantle-Shell Preparation and Ca45. *Biological Bulletin*, *104*(3), 398–407. https://doi.org/10.2307/1538493

Kamphausen, L., Jensen, A., & Hawkins, L. (2011). Unusually High Proportion of Males in a Collapsing Population of Commercially Fished Oysters (Ostrea edulis) in the Solent, United Kingdom. *Journal of Shellfish Research*, *30*(2), 217–222. https://doi.org/10.2983/035.030.0204

Kharouba, H. M., & Wolkovich, E. M. (2020). Disconnects between ecological theory and data in phenological mismatch research. *Nature Climate Change*, *10*(5), Article 5. https://doi.org/10.1038/s41558-020-0752-x

Kızılkaya, B., Yıldız, H., & Vural, P. (2024). Shell Composition Analysis of European Flat Oyster (Ostrea edulis, Linnaeus 1758) From Marmara Sea, Türkiye: Insights Into Chemical Properties. *Marine Science and Technology Bulletin*, *13*(2), Article 2. https://doi.org/10.33714/masteb.1493896

Klotter, K. (1960). General properties of oscillating systems. *Cold Spring Harbor Symposia on Quantitative Biology*, *25*, 185–187. https://doi.org/10.1101/sqb.1960.025.01.016

Konopka, R. J., & Benzer, S. (1971). Clock mutants of Drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America*, *68*(9), 2112–2116. https://doi.org/10.1073/pnas.68.9.2112

Korringa, P. (1940). *Experiments End Observations on Swarming, Pelagic Life and Setting in the European Flat Oyster, Ostrea Edulis L.: Contribution from the Governmental Institution for Biological Fishery Research*. s.e.

Korringa, P. (1947). Relations between the Moon and Periodicity in the Breeding of Marine Animals. *Ecological Monographs*, *17*(3), 347–381. https://doi.org/10.2307/1948665

Kramer, G. (1950). Weitere Analyse der Faktoren, welche die Zugaktivität des gekäfigten Vogels orientieren. *Naturwissenschaften*, *37*(16), 377–378. https://doi.org/10.1007/BF00626007

Krittika, S., & Yadav, P. (2020). Circadian clocks: An overview on its adaptive significance. *Biological Rhythm Research*, *51*(7), 1109–1132. https://doi.org/10.1080/09291016.2019.1581480

Kronfeld-Schor, N., & Dayan, T. (2003). Partitioning of Time as an Ecological Resource. *Annual Review of Ecology, Evolution, and Systematics*, *34*(Volume 34, 2003), 153–181. https://doi.org/10.1146/annurev.ecolsys.34.011802.132435

Kronfeld-Schor, N., Dominoni, D., de la Iglesia, H., Levy, O., Herzog, E. D., Dayan, T., & Helfrich-Forster, C. (2013). Chronobiology by moonlight. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1765), 20123088. https://doi.org/10.1098/rspb.2012.3088

Küfeoğlu, S. (2022). SDG-14: Life Below Water. In S. Küfeoğlu (Ed.), *Emerging Technologies: Value Creation for Sustainable Development* (pp. 453–468). Springer International Publishing. https://doi.org/10.1007/978-3-031-07127-0_16

Kyba, C. C. M., Altıntaş, Y. Ö., Walker, C. E., & Newhouse, M. (2023). Citizen scientists report global rapid reductions in the visibility of stars from 2011 to 2022. *Science (New York, N.Y.)*, *379*(6629), 265–268. https://doi.org/10.1126/science.abq7781

Lapegue, S., Beaumont, A., Boudry, P., Goulletquer, P., Boudry, P., & Goulletquer, P. (2006, June 12). *European flat oyster—Ostrea edulis*. GENIMPACT - Evaluation of genetic impact of aquaculture activies on native population.

A European network, WP1 workshop "Genetics of domestication, breeding and enhancement of performance of fish and shellfish. https://archimer.ifremer.fr/doc/00000/3321/

Last, K. S., Bailhache, T., Kramer, C., Kyriacou, C. P., Rosato, E., & Olive, P. J. W. (2009). Tidal, daily, and lunar-day activity cycles in the marine polychaete Nereis virens. *Chronobiology International*, *26*(2), 167–183. https://doi.org/10.1080/07420520902774524

Le Moal, A., Payton, L., Andrade, H., Camus, L., Ballantine, C., Ciret, P., & Tran, D. (2023). Blue mussels' valve behavior exhibits daily and lunar rhythms during the high Arctic polar day. *Marine Biology*, *170*(9), 113. https://doi.org/10.1007/s00227-023-04257-6

Le Moal, A., Payton, L., Geerebaert, Y., Meyer, B., Pogoda, B., & Tran, D. (2025). *Valve behavior of the European flat oyster Ostrea edulis and associated underwater sound and temperature data, recorded during a 18-month in situ experiment in Helgoland, Germany* [Dataset bundled publication]. PANGAEA. https://doi.pangaea.de/10.1594/PANGAEA.983652

Le Moal, A., Tran, D., Payton, L., Pogoda, B., & Meyer, B. (2025). Characterization of the daily and circadian valve behavior of the European flat oyster Ostrea edulis. *Scientific Reports*, *15*(1), 14952. https://doi.org/10.1038/s41598-025-98746-x

Le Moullac, G., Quéau, I., Le Souchu, P., Pouvreau, S., Moal, J., René Le Coz, J., & François Samain, J. (2007). Metabolic adjustments in the oyster Crassostrea gigas according to oxygen level and temperature. *Marine Biology Research*, *3*(5), 357–366. https://doi.org/10.1080/17451000701635128

Ledoux, T., Clements, J. C., Comeau, L. A., Cervello, G., Tremblay, R., Olivier, F., Chauvaud, L., Bernier, R. Y., & Lamarre, S. G. (2023). Effects of anthropogenic sounds on the behavior and physiology of the Eastern oyster (Crassostrea virginica). *Frontiers in Marine Science*, *10*. https://doi.org/10.3389/fmars.2023.1104526

Levitan, D. R., & Petersen, C. (1995). Sperm limitation in the sea. *Trends in Ecology & Evolution*, *10*(6), 228–231. https://doi.org/10.1016/S0169-5347(00)89071-0

Liberman, R., Fine, M., & Benayahu, Y. (2021). Simulated climate change scenarios impact the reproduction and early life stages of a soft coral. *Marine Environmental Research*, *163*, 105215. https://doi.org/10.1016/j.marenvres.2020.105215

Lin, F.-J., Song, W., Meyer-Bernstein, E., Naidoo, N., & Sehgal, A. (2001). Photic Signaling by Cryptochrome in the Drosophila Circadian System. *Molecular and Cellular Biology*, *21*(21), 7287–7294. https://doi.org/10.1128/MCB.21.21.7287-7294.2001

Linnaeus, C., & Freer, S. (2003). *Linnaeus' Philosophia Botanica*. Oxford University Press.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods (San Diego, Calif.)*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Loosanoff, V. L., & Davis, H. C. (1963). *Rearing of Bivalve Mollusks*. *1*, 1–136. https://doi.org/10.1016/S0065-2881(08)60257-6

Lubet, p. (1991). Reproduction des mollusques [Reproduction of molluscs]. In *Bases Biologiques et Ecologiques de l'Aquaculture* (pp. 168–204).

Maneiro, V., Silva, A., Pazos, A. J., Sánchez, J. L., & Pérez-Parallé, M. L. (2017). Effects of temperature and photoperiod on the conditioning of the flat oyster (Ostrea edulis L.) in autumn. *Aquaculture Research*, *48*(8), 4554–4562. https://doi.org/10.1111/are.13280

Mardones-Toledo, D. A., Montory, J. A., Joyce, A., Thompson, R. J., Diederich, C. M., Pechenik, J. A., Mardones, M. L., & Chaparro, O. R. (2015). Brooding in the Chilean Oyster Ostrea chilensis: Unexpected Complexity in the Movements of Brooded Offspring within the Mantle Cavity. *PLOS ONE*, *10*(4), e0122859. https://doi.org/10.1371/journal.pone.0122859

Marescaux, J., Falisse, E., Lorquet, J., Van Doninck, K., Beisel, J.-N., & Descy, J.-P. (2016). Assessing filtration rates of exotic bivalves: Dependence on algae concentration and seasonal factors. *Hydrobiologia*, *777*(1), 67–78. https://doi.org/10.1007/s10750-016-2764-0

Marteil, L. (1976). La conchyliculture francaise. 2eme partie. Biologie de l'huitre et de la moule. *Revue Des Travaux de l'Institut Des Pêches Maritimes*, *40*(2), 149–346.

Mat, A. M., Massabuau, J.-C., Ciret, P., & Tran, D. (2012). Evidence for a Plastic Dual Circadian Rhythm in the Oyster Crassostrea gigas. *Chronobiology International*, *29*(7), 857–867. https://doi.org/10.3109/07420528.2012.699126

Mat, A. M., Perrigault, M., Massabuau, J.-C., & Tran, D. (2016). Role and expression of cry1 in the adductor muscle of the oyster Crassostrea gigas during daily and tidal valve activity rhythms. *Chronobiology International*, *33*(8), 949–963. https://doi.org/10.1080/07420528.2016.1181645

Mat, A., Massabuau, J.-C., Ciret, P., & Tran, D. (2014). Looking for the clock mechanism responsible for circatidal behavior in the oyster Crassostrea gigas. *Marine Biology*, *161*(1), 89–99. https://doi.org/10.1007/s00227-013-2317-2

Matsumoto, A., Ukai-Tadenuma, M., Yamada, R. G., Houl, J., Uno, K. D., Kasukawa, T., Dauwalder, B., Itoh, T. Q., Takahashi, K., Ueda, R., Hardin, P. E., Tanimura, T., & Ueda, H. R. (2007). A functional genomics strategy reveals clockwork orange as a transcriptional regulator in the Drosophila circadian clock. *Genes & Development*, *21*(13), 1687–1700. https://doi.org/10.1101/gad.1552207

Mercier, A., & Hamel, J.-F. (2010). Synchronized breeding events in sympatric marine invertebrates: Role of behavior and fine temporal windows in maintaining reproductive isolation. *Behavioral Ecology and Sociobiology*, *64*(11), 1749–1765. https://doi.org/10.1007/s00265-010-0987-z

Merk, V., Colsoul, B., & Pogoda, B. (2020). Return of the native: Survival, growth and condition of European oysters reintroduced to German offshore waters. *Aquatic Conservation Marine and Freshwater Ecosystems*, *30*, 2180–2190. https://doi.org/10.1002/aqc.3426

Mermet, J., Yeung, J., & Naef, F. (2017). Systems Chronobiology: Global Analysis of Gene Regulation in a 24-Hour Periodic World. *Cold Spring Harbor Perspectives in Biology*, *9*(3), a028720. https://doi.org/10.1101/cshperspect.a028720

Möbius, K. A. (1877). *Die Auster und die Austernwirthschaft*. Verlag von Wiegandt, Hemple & Parey.

Monecke, S. (2024). Threatened chronotopes: Can chronobiology help endangered species? *Journal of Comparative Physiology A*, *210*(4), 717–733. https://doi.org/10.1007/s00359-024-01692-8

Moore-Ede, M. C. (1986). Physiology of the circadian timing system: Predictive versus reactive homeostasis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *250*(5), R737–R752. https://doi.org/10.1152/ajpregu.1986.250.5.R737

Morga, B., Arzul, I., Faury, N., & Renault, T. (2010). Identification of genes from flat oyster *Ostrea edulis* as suitable housekeeping genes for quantitative real time PCR. *Fish & Shellfish Immunology*, *29*(6), 937–945. https://doi.org/10.1016/j.fsi.2010.07.028

Morton, B. S. (1977). The tidal rhythm of feeding and digestion in the pacific oyster, *Crassostrea gigas* (Thunberg). *Journal of Experimental Marine Biology and Ecology*, *26*(2), 135–151. https://doi.org/10.1016/0022-0981(77)90103-4

Müller, K. (1978). The flexibility of the circadian system of fish at different latitudes. *Rhythmic Activity of Fishes*, 91–104.

Mure, L. S., Le, H. D., Benegiamo, G., Chang, M. W., Rios, L., Jillani, N., Ngotho, M., Kariuki, T., Dkhissi-Benyahya, O., Cooper, H. M., & Panda, S. (2018). Diurnal transcriptome atlas of a primate across major neural and peripheral tissues. *Science*, *359*(6381), eaao0318. https://doi.org/10.1126/science.aao0318

Nangle, S. N., Rosensweig, C., Koike, N., Tei, H., Takahashi, J. S., Green, C. B., & Zheng, N. (2014). Molecular assembly of the period-cryptochrome circadian transcriptional repressor complex. *eLife*, *3*, e03674. https://doi.org/10.7554/eLife.03674

Nawathean, P., & Rosbash, M. (2004). The Doubletime and CKII Kinases Collaborate to Potentiate Drosophila PER Transcriptional Repressor Activity. *Molecular Cell*, *13*(2), 213–223. https://doi.org/10.1016/S1097-2765(03)00503-3

Naylor, E. (1999). Marine Animal Behaviour In Relation To Lunar Phase. *Earth, Moon, and Planets*, *85*(0), 291–302. https://doi.org/10.1023/A:1017088504226

Naylor, E. (2010). *Chronobiology of Marine Organisms*. Cambridge University Press. https://doi.org/10.1017/CBO9780511803567

Nelson, T. C. (1928). Relation of Spawning of the Oyster to Temperature. *Ecology*, *9*(2), 145–154. https://doi.org/10.2307/1929351

Nelson, T. C., & Allison, J. B. (1940). On the nature and action of diantlin; a new hormone-like substance carried by the spermatozoa of the oyster. *Journal of Experimental Zoology*, *85*(2), 299–338. https://doi.org/10.1002/jez.1400850207

Ó Foighil, D., & Taylor, D. J. (2000). Evolution of Parental Care and Ovulation Behavior in Oysters. *Molecular Phylogenetics and Evolution*, *15*(2), 301–313. https://doi.org/10.1006/mpev.1999.0755

Oliphant, A., Sia, C. Y., Kyriacou, C. P., Wilcockson, D. C., & Hastings, M. H. (2025). Expression of clock genes tracks daily and tidal time in brains of intertidal crustaceans Eurydice pulchra and Parhyale hawaiensis. *Current Biology*, *35*(12), 2802-2815.e5. https://doi.org/10.1016/j.cub.2025.04.047

Olsen, O. T. (1883). *The piscatorial atlas of the North Sea, English and St. George's Channels, illustrating the fishing ports, boats, gear, species of fish (how, where, and when caught), and other information concerning fish and fisheries*. https://www.waterbouwkundiglaboratorium.be/nl/catalogus

Oren, M., Tarrant, A. M., Alon, S., Simon-Blecher, N., Elbaz, I., Appelbaum, L., & Levy, O. (2015). Profiling molecular and behavioral circadian rhythms in the non-symbiotic sea anemone Nematostella vectensis. *Scientific Reports*, *5*(1), 11418. https://doi.org/10.1038/srep11418

Orton, J. H. (1927). Observation and Experiments on Sex-Change in the European Oyster (O. edulis.): Part I. The Change from Female to Male. *Journal of the Marine Biological Association of the United Kingdom*, *14*(4), 967–1045. https://doi.org/10.1017/S0025315400051183

Orton, J. H. (1937). *Oyster Biology and Oyster-culture: Being the Buckland Lectures for 1935*. E. Arnold & Company.

OSPAR. (2009). *Background document for Ostrea edulis and Ostrea edulis beds*. In Biodiversity Series 428. Edited by Jan Haelters and Francis Kerckhof. Paris: OSPAR Commission, 22p.

OSPAR. (2010). The North-East Atlantic Environment Strategy-Strategy of the OSPAR Commission for the Protection of the Marine Environment of the North-East Atlantic 2010–2020. *Paris: OSPAR Commission*, 1–27.

Öztürk, N., Song, S.-H., Özgür, S., Selby, C. P., Morrison, L., Partch, C., Zhong, D., & Sancar, A. (2007). Structure and Function of Animal Cryptochromes. *Cold Spring Harbor Symposia on Quantitative Biology*, *72*, 119–131. https://doi.org/10.1101/sqb.2007.72.015

Palanques, A., Guillén, J., & Puig, P. (2001). Impact of bottom trawling on water turbidity and muddy sediment of an unfished continental shelf. *Limnology and Oceanography*, *46*(5), 1100–1110. https://doi.org/10.4319/lo.2001.46.5.1100

Palmer, J. D. (1995). Review of the Dual-Clock Control of Tidal Rhythms and the Hypothesis that the Same Clock Governs Both Circatidal and Circadian Rhythms. *Chronobiology International*, *12*(5), 299–310. https://doi.org/10.3109/07420529509057279

Palmer, J. D. (2000). The clocks controlling the tide-associated rhythms of intertidal animals. *BioEssays*, *22*(1), 32–37. https://doi.org/10.1002/(SICI)1521-1878(200001)22:1<32::AID-BIES7>3.0.CO;2-U

Palmer, J. D. (2002). *The Living Clock: The Orchestrator of Biological Rhythms*. Oxford University Press.

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(Volume 37, 2006), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Partch, C. L., Green, C. B., & Takahashi, J. S. (2014). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology*, *24*(2), 90–99. https://doi.org/10.1016/j.tcb.2013.07.002

Payton, L., Last, K. S., Grigor, J., Noirot, C., Hüppe, L., Conway, D. V. P., Dannemeyer, M., Wilcockson, D., & Meyer, B. (2024). Revealing the profound influence of diapause on gene expression: Insights from the annual transcriptome of the copepod Calanus finmarchicus. *Molecular Ecology*, *33*(13), e17425. https://doi.org/10.1111/mec.17425

Payton, L., Perrigault, M., Hoede, C., Massabuau, J.-C., Sow, M., Huvet, A., Boullot, F., Fabioux, C., Hegaret, H., & Tran, D. (2017). Remodeling of the cycling transcriptome of the oyster Crassostrea gigas by the harmful algae Alexandrium minutum. *Scientific Reports*, *7*(1), 3480. https://doi.org/10.1038/s41598-017-03797-4

Payton, L., Sow, M., Massabuau, J.-C., Ciret, P., & Tran, D. (2017). How annual course of photoperiod shapes seasonal behavior of diploid and triploid oysters, Crassostrea gigas. *PLOS ONE*, *12*(10), e0185918. https://doi.org/10.1371/journal.pone.0185918

Payton, L., & Tran, D. (2019). Moonlight cycles synchronize oyster behaviour. *Biology Letters*, *15*(1), 20180299. https://doi.org/10.1098/rsbl.2018.0299

Peirson, S., & Foster, R. G. (2006). Melanopsin: Another Way of Signaling Light. *Neuron*, *49*(3), 331–339. https://doi.org/10.1016/j.neuron.2006.01.006

Peng, C., Zhao, X., & Liu, G. (2015). Noise in the Sea and Its Impacts on Marine Organisms. *International Journal of Environmental Research and Public Health*, *12*(10), Article 10. https://doi.org/10.3390/ijerph121012304

Per Huffeldt, N. (2020). Photic Barriers to Poleward Range-shifts. *Trends in Ecology & Evolution*, *35*(8), 652–655. https://doi.org/10.1016/j.tree.2020.04.011

Perrigault, M., Andrade, H., Bellec, L., Ballantine, C., Camus, L., & Tran, D. (2020). Rhythms during the polar night: Evidence of clock-gene oscillations in the Arctic scallop Chlamys islandica. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1933), 20201001. https://doi.org/10.1098/rspb.2020.1001

Perrigault, M., & Tran, D. (2017). Identification of the Molecular Clockwork of the Oyster Crassostrea gigas. *PLOS ONE*, *12*(1), e0169790. https://doi.org/10.1371/journal.pone.0169790

Peschel, N., Chen, K. F., Szabo, G., & Stanewsky, R. (2009). Light-Dependent Interactions between the Drosophila Circadian Clock Factors Cryptochrome, Jetlag, and Timeless. *Current Biology*, *19*(3), 241–247. https://doi.org/10.1016/j.cub.2008.12.042

Piccolin, F., Pitzschler, L., Biscontin, A., Kawaguchi, S., & Meyer, B. (2020). Circadian regulation of diel vertical migration (DVM) and metabolism in Antarctic krill Euphausia superba. *Scientific Reports*, *10*(1), 16796. https://doi.org/10.1038/s41598-020-73823-5

Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in drosophila. *Proceedings of the National Academy of Sciences*, *40*(10), 1018–1029. https://doi.org/10.1073/pnas.40.10.1018

Pittendrigh, C. S. (1960). Circadian Rhythms and the Circadian Organization of Living Systems. *Cold Spring Harbor Symposia on Quantitative Biology*, *25*, 159–184. https://doi.org/10.1101/SQB.1960.025.01.015

Pogoda, B. (2019). Current Status of European Oyster Decline and Restoration in Germany. *Humanities*, *8*(1), Article 1. https://doi.org/10.3390/h8010009

Pogoda, B., Boudry, P., Bromley, C., Cameron, T. C., Colsoul, B., Donnan, D., Hancock, B., Hugh-Jones, T., Preston, J., Sanderson, W. G., Sas, H., Brown, J., Bonacic, K., von Nordheim, H., & zu Ermgassen, P. S. E. (2020). NORA moving forward: Developing an oyster restoration network in Europe to support the Berlin Oyster Recommendation. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2031–2037. https://doi.org/10.1002/aqc.3447

Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., Sanderson, W., & Nordheim, H. von. (2019a). The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation: Bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquatic Living Resources*, *32*, 13. https://doi.org/10.1051/alr/2019012

Pogoda, B., Brown, J., Hancock, B., Preston, J., Pouvreau, S., Kamermans, P., Sanderson, W., & Nordheim, H. von. (2019b). The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation: Bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquatic Living Resources*, *32*, 13. https://doi.org/10.1051/alr/2019012

Pogoda, B., Hausen, T., Rothe, M., Bakker, F., Hauser, S., Colsoul, B., Dureuil, M., Krause, J., Heinicke, K., Pusch, C., Eisenbarth, S., Kreutle, A., Peter, C., & Pesch, R. (2023). Come, tell me how you live: Habitat suitability analysis for Ostrea edulis restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *33*(7), 678–695. https://doi.org/10.1002/aqc.3928

Pogoda, B., Merk, V., Colsoul, B., Hausen, T., Peter, C., Pesch, R., Kramer, M., Jaklin, S., Holler, P., Bartholomä, A., Michaelis, R., & Prinz, K. (2020). Site selection for biogenic reef restoration in offshore environments: The Natura 2000 area Borkum Reef Ground as a case study for native oyster restoration. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2163–2179. https://doi.org/10.1002/aqc.3405

Pourmozaffar, S., Tamadoni Jahromi, S., Rameshi, H., Sadeghi, A., Bagheri, T., Behzadi, S., Gozari, M., Zahedi, M. R., & Abrari Lazarjani, S. (2020). The role of salinity in physiological responses of bivalves. *Reviews in Aquaculture*, *12*(3), 1548–1566. https://doi.org/10.1111/raq.12397

Pouvreau, S., Lapègue, S., Arzul, I., & Boudry, P. (2023). Fifty years of research to counter the decline of the European flat oyster (Ostrea edulis): A review of French achievements and prospects for the restoration of remaining beds and revival of aquaculture production. *Aquatic Living Resources*, *36*, 13. https://doi.org/10.1051/alr/2023006

Preston, J., zu Ermgassen, P., Fabra, M., Lee, H., Rodriguez Perez, A., Hayden-Hughes, M., Colsoul, B., Strand, Å., Ashton, E., Gamble, C., Hancock, B., Sanderson, W., Thurstan, R., Bromley, C., Fariñas-Franco, J., Bonacic, K., Frankic, A., Mcafee, D., & Helmer, L. (2020). *European Native Oyster Habitat Restoration Handbook UK & Ireland*.

Prytherch, H. F. (1929). *Investigation of the Physical Conditions Controlling Spawning of Oysters and the Occurrence, Distribution, and Setting of Oyster Larvae in Milford Harbor, Connecticut*. U.S. Government Printing Office.

R Core Team. (2023). *R: A language and environment for statistical computing*. [Computer software]. Foundation for Statistical Computing. URL https://www.R-project.org/

Ramos, A. P., Gustafsson, O., Labert, N., Salecker, I., Nilsson, D.-E., & Averof, M. (2019). Analysis of the genetically tractable crustacean Parhyale hawaiensis reveals the organisation of a sensory system for low-resolution vision. *BMC Biology*, *17*(1), 67. https://doi.org/10.1186/s12915-019-0676-y

Reebs, S. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries*, *12*, 349–371.

Reiter, R. J. (1993). The melatonin rhythm: Both a clock and a calendar. *Experientia*, *49*(8), 654–664. https://doi.org/10.1007/BF01923947

Relógio, A., Westermark, P. O., Wallach, T., Schellenberg, K., Kramer, A., & Herzel, H. (2011). Tuning the Mammalian Circadian Clock: Robust Synergy of Two Loops. *PLOS Computational Biology*, *7*(12), e1002309. https://doi.org/10.1371/journal.pcbi.1002309

Reppert, S. M., & Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature*, *418*(6901), 935–941. https://doi.org/10.1038/nature00965

Rey, G. (2011). *On the Relationship Between Protein-DNA Interactions and Circadian Gene Expression in Mouse Liver*.

Rice, P., Ray, S. M., Painter, S. J., & Nagle, G. T. (2002). An intrinsic membrane protein in oyster sperm stimulates spawning behaviors in Crassostrea virginica: Implications for aquaculture. *Journal of Shellfish Research*, *21*, 715–718.

Roberts, L., Cheesman, S., Breithaupt, T., & Elliott, M. (2015). Sensitivity of the mussel Mytilus edulis to substrate-borne vibration in relation to anthropogenically generated noise. *Marine Ecology Progress Series*, *538*, 185–195. https://doi.org/10.3354/meps11468

Rock, A., Wilcockson, D., & Last, K. S. (2022). Towards an Understanding of Circatidal Clocks. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.830107

Roenneberg, T., & Merrow, M. (2005). Circadian clocks—The fall and rise of physiology. *Nature Reviews. Molecular Cell Biology*, *6*(12), 965–971. https://doi.org/10.1038/nrm1766

Röthig, T., Trevathan-Tackett, S. M., Voolstra, C. R., Ross, C., Chaffron, S., Durack, P. J., Warmuth, L. M., & Sweet, M. (2023). Human-induced salinity changes impact marine organisms and ecosystems. *Global Change Biology*, *29*(17), 4731–4749. https://doi.org/10.1111/gcb.16859

Ruiz, C., Martinez, D., Mosquera, G., Abad, M., & Sánchez, J. L. (1992). Seasonal variations in condition, reproductive activity and biochemical composition of the flat oyster, Ostrea edulis, from San Cibran (Galicia, Spain). *Marine Biology*, *112*(1), 67–74. https://doi.org/10.1007/BF00349729

Sáenz de Miera, C., Monecke, S., Bartzen-Sprauer, J., Laran-Chich, M.-P., Pévet, P., Hazlerigg, D. G., & Simonneaux, V. (2014). A Circannual Clock Drives Expression of Genes Central for Seasonal Reproduction. *Current Biology*, *24*(13), 1500–1506. https://doi.org/10.1016/j.cub.2014.05.024

Sanders, D., Frago, E., Kehoe, R., Patterson, C., & Gaston, K. J. (2021). A meta-analysis of biological impacts of artificial light at night. *Nature Ecology & Evolution*, *5*(1), 74–81. https://doi.org/10.1038/s41559-020-01322-x

Sas, H., Deden, B., Kamermans, P., zu Ermgassen, P. S., Pogoda, B., Preston, J., Helmer, L., Holbrook, Z., Arzul, I., Have, T. van der, Villalba, A., Colsoul, B., Lown, A., Merk, V., Zwerschke, N., & Reuchlin, E. (2020). Bonamia infection in native oysters (Ostrea edulis) in relation to European restoration projects. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2150–2162. https://doi.org/10.1002/aqc.3430

Scargle, J. D. (1982). Studies in astronomical time series analysis. II. Statistical aspects of spectral analysis of unevenly spaced data. *The Astrophysical Journal*, *263*, 835–853. https://doi.org/10.1086/160554

Schmalen, I., Reischl, S., Wallach, T., Klemz, R., Grudziecki, A., Prabu, J. R., Benda, C., Kramer, A., & Wolf, E. (2014). Interaction of Circadian Clock Proteins CRY1 and PER2 Is Modulated by Zinc Binding and Disulfide Bond Formation. *Cell*, *157*(5), 1203–1215. https://doi.org/10.1016/j.cell.2014.03.057

Sharma, V. K., & Chandrashekaran, M. K. (2005). Zeitgebers (time cues) for biological clocks. *Current Science*, *89*(7), 1136–1146.

Shearman, L. P., Sriram, S., Weaver, D. R., Maywood, E. S., Chaves, I., Zheng, B., Kume, K., Lee, C. C., van der Horst, G. T., Hastings, M. H., & Reppert, S. M. (2000). Interacting molecular loops in the mammalian circadian clock. *Science (New York, N.Y.)*, *288*(5468), 1013–1019. https://doi.org/10.1126/science.288.5468.1013

Shumway, S. E. (1977). Effect of salinity fluctuation on the osmotic pressure and Na+, Ca2+ and Mg2+ ion concentrations in the hemolymph of bivalve molluscs. *Marine Biology*, *41*(2), 153–177. https://doi.org/10.1007/BF00394023

Smyth, D., & Roberts, D. (2010). The European oyster (Ostrea edulis) and its epibiotic succession. *Hydrobiologia*, *655*(1), 25–36. https://doi.org/10.1007/s10750-010-0401-x

Smyth, T. J., Wright, A. E., McKee, D., Tidau, S., Tamir, R., Dubinsky, Z., Iluz, D., & Davies, T. W. (2021). A global atlas of artificial light at night under the sea. *Elementa: Science of the Anthropocene*, *9*(1), 00049. https://doi.org/10.1525/elementa.2021.00049

Spärck, R. (1925). Studies on the biology of the oyster (Ostrea edulis) in the Limfjord with special reference to the influence of temperature on the sex change. *In Commission by GEC Gad.*

Stanewsky, R., Kaneko, M., Emery, P., Beretta, B., Wager-Smith, K., Kay, S. A., Rosbash, M., & Hall, J. C. (1998). The cryb Mutation Identifies Cryptochrome as a Circadian Photoreceptor in Drosophila. *Cell*, *95*(5), 681–692. https://doi.org/10.1016/S0092-8674(00)81638-4

Stanton, D., Justin, H. S., & Reitzel, A. M. (2022). Step in Time: Conservation of Circadian Clock Genes in Animal Evolution. *Integrative and Comparative Biology*, *62*(6), 1503–1518. https://doi.org/10.1093/icb/icac140

Starr, M., Himmelman, J. H., & Therriault, J. C. (1990). Direct coupling of marine invertebrate spawning with phytoplankton blooms. *Science (New York, N.Y.)*, *247*(4946), 1071–1074. https://doi.org/10.1126/science.247.4946.1071

Sun, X. J., Zhou, L. Q., Tian, J. T., Liu, Z. H., Wu, B., Dong, Y. H., Yang, A. G., & Ma, W. M. (2016). Transcriptome survey of phototransduction and clock genes in marine bivalves. *Genetics and Molecular Research: GMR*, *15*(4). https://doi.org/10.4238/gmr15048726

Suquet, M., Pouvreau, S., Queau, I., Boulais, M., Grand, J. L., Ratiskol, D., & Cosson, J. (2018). Biological characteristics of sperm in European flat oyster (Ostrea edulis). *Aquatic Living Resources*, *31*, 20. https://doi.org/10.1051/alr/2018008

Sweeney, A., Boch, C., Johnsen, S., & Morse, D. (2011). Twilight spectral dynamics and the coral reef invertebrate spawning response. *The Journal of Experimental Biology*, *214*, 770–777. https://doi.org/10.1242/jeb.043406

Teschke, K., Karez, R., Schubert, P. R., & Beermann, J. (2020). Colonisation success of introduced oysters is driven by wave-related exposure. *Biological Invasions*, *22*(7), 2121–2127. https://doi.org/10.1007/s10530-020-02246-0

Tessmar-Raible, K., Raible, F., & Arboleda, E. (2011). Another place, another timer: Marine species and the rhythms of life. *BioEssays*, *33*(3), 165–172. https://doi.org/10.1002/bies.201000096

Thaben, P. F., & Westermark, P. O. (2014). Detecting Rhythms in Time Series with RAIN. *Journal of Biological Rhythms*, *29*(6), 391–400. https://doi.org/10.1177/0748730414553029

Thomas, S., Collins, K., Hauton, C., & Jensen, A. (2022). A Review of the Ecosystem Services Provided by the Native Oyster (Ostrea edulis): Implications for Restoration. *IOP Conference Series: Materials Science and Engineering*, *1245*(1), 012010. https://doi.org/10.1088/1757-899X/1245/1/012010

Thurstan, R. H., McCormick, H., Preston, J., Ashton, E. C., Bennema, F. P., Cetinić, A. B., Brown, J. H., Cameron, T. C., Costa, F. da, Donnan, D., Ewers, C., Fortibuoni, T., Galimany, E., Giovanardi, O., Grancher, R., Grech, D., Hayden-Hughes, M., Helmer, L., Jensen, K. T., zu Ermgassen, P. S. (2023). *Historical dataset details the distribution, extent and form of lost Ostrea edulis reef ecosystems.* https://ecoevorxiv.org/repository/view/6359/

Thurstan, R. H., McCormick, H., Preston, J., Ashton, E. C., Bennema, F. P., Cetinić, A. B., Brown, J. H., Cameron, T. C., da Costa, F., Donnan, D. W., Ewers, C., Fortibuoni, T., Galimany, E., Giovanardi, O., Grancher, R., Grech, D., Hayden-Hughes, M., Helmer, L., Jensen, K. T., zu Ermgassen, P. S. E. (2024). Records reveal the vast historical extent of European oyster reef ecosystems. *Nature Sustainability*, 1–11. https://doi.org/10.1038/s41893-024-01441-4

Tidau, S., & Briffa, M. (2016). Review on behavioral impacts of aquatic noise on crustaceans. *Proceedings of Meetings on Acoustics*, *27*(1), 010028. https://doi.org/10.1121/2.0000302

Tonk, L., Witbaard, R., Dalen, P. van, Cheng, C. H., & Kamermans, P. (2023). Applicability of the gape monitor to study flat oyster (Ostrea edulis) feeding behaviour. *Aquatic Living Resources*, *36*(6), 6. https://doi.org/10.1051/alr/2022021

Tosches, M. A., Bucher, D., Vopalensky, P., & Arendt, D. (2014). Melatonin Signaling Controls Circadian Swimming Behavior in Marine Zooplankton. *Cell*, *159*(1), 46–57. https://doi.org/10.1016/j.cell.2014.07.042

Tran, D., Andrade, H., Camus, L., Leopold, P., Ballantine, C., Berge, J., Durier, G., Sow, M., & Ciret, P. (2023). Evidence of separate influence of moon and sun on light synchronization of mussel's daily rhythm during the polar night. *iScience*, *26*(3). https://doi.org/10.1016/j.isci.2023.106168

Tran, D., Andrade, H., Durier, G., Ciret, P., Leopold, P., Sow, M., Ballantine, C., Camus, L., Berge, J., & Perrigault, M. (2020). Growth and behaviour of blue mussels, a re-emerging polar resident, follow a strong annual rhythm shaped by the extreme high Arctic light regime. *Royal Society Open Science*, *7*(10), 200889. https://doi.org/10.1098/rsos.200889

Tran, D., Nadau, A., Durrieu, G., Ciret, P., Parisot, J.-P., & Massabuau, J.-C. (2011). Field Chronobiology of a Molluscan Bivalve: How the Moon and Sun Cycles Interact to Drive Oyster Activity Rhythms. *Chronobiology International*, *28*(4), 307–317. https://doi.org/10.3109/07420528.2011.565897

Tran, D., Perrigault, M., Ciret, P., & Payton, L. (2020). Bivalve mollusc circadian clock genes can run at tidal frequency. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1918), 20192440. https://doi.org/10.1098/rspb.2019.2440

Tran, D., Sow, M., Camus, L., Ciret, P., Berge, J., & Massabuau, J.-C. (2016). In the darkness of the polar night, scallops keep on a steady rhythm. *Scientific Reports*, *6*(1), 32435. https://doi.org/10.1038/srep32435

Tuttle-Raycraft, S., & Ackerman, J. D. (2019). Living the high turbidity life: The effects of total suspended solids, flow, and gill morphology on mussel feeding. *Limnology and Oceanography*, *64*(6), 2526–2537. https://doi.org/10.1002/lno.11202

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*, *3*(7), research0034.1. https://doi.org/10.1186/gb-2002-3-7-research0034

Visser, M. E., Caro, S. P., van Oers, K., Schaper, S. V., & Helm, B. (2010). Phenology, seasonal timing and circannual rhythms: Towards a unified framework. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3113–3127. https://doi.org/10.1098/rstb.2010.0111

Walne, P. (1974). *Culture of Bivalve Molluscs: 50 Years Experience at Conwy*. Fishing News Books Limited, Surrey.

Waltham, N. J., Elliott, M., Lee, S. Y., Lovelock, C., Duarte, C. M., Buelow, C., Simenstad, C., Nagelkerken, I., Claassens, L., Wen, C. K.-C., Barletta, M., Connolly, R. M., Gillies, C., Mitsch, W. J., Ogburn, M. B., Purandare, J., Possingham, H., & Sheaves, M. (2020). UN Decade on Ecosystem Restoration 2021–2030—What Chance for Success in Restoring Coastal Ecosystems? *Frontiers in Marine Science*, *7*. https://doi.org/10.3389/fmars.2020.00071

Wanninger, A., & Wollesen, T. (2019). The evolution of molluscs. *Biological Reviews*, *94*(1), 102–115. https://doi.org/10.1111/brv.12439

Watson, G., Bentley, M., Gaudron, S., & Hardege, J. (2003). The role of chemical signals in the spawning induction of polychaete worms and other marine invertebrates. *Journal of Experimental Marine Biology and Ecology*, *294*, 169–187. https://doi.org/10.1016/S0022-0981(03)00264-8

Webb, H. M. (1976). Interactions of daily and tidal rhythms. Biological rhythms in the marine environment. *University of South Carolina Press, Columbia*, 129–135.

Whymper, F. (1884). *The fisheries of the world: An illustrated and descriptive record of the International fisheries exhibition, 1883*. Cassell & Company, Limited.

Williams, B. G., Palmer, J. D., & Hutchinson, D. N. (1993). Comparative studies of tidal rhythms XIII. Is a clam clock similar to those of other intertidal animals? *Marine Behaviour and Physiology*, *24*(1), 1–14. https://doi.org/10.1080/10236249309378873

Williams, B. G., & Pilditch, C. A. (1997). The Entrainment of Persistent Tidal Rhythmicity in a Filter-Feeding Bivalve Using Cycles of Food Availability. *Journal of Biological Rhythms*, *12*(2), 173–181. https://doi.org/10.1177/074873049701200208

Wilson, J. H., & Simons, J. (1985). Gametogenesis and breeding of *Ostrea edulis* on the west coast of Ireland. *Aquaculture*, *46*(4), 307–321. https://doi.org/10.1016/0044-8486(85)90109-7

Yerushalmi, S., & Green, R. M. (2009). Evidence for the adaptive significance of circadian rhythms. *Ecology Letters*, *12*(9), 970–981. https://doi.org/10.1111/j.1461-0248.2009.01343.x

Yonge, C. M. (1926). Structure and Physiology of the Organs of Feeding and Digestion in Ostrea edulis. *Journal of the Marine Biological Association of the United Kingdom*, *14*(2), 295–386. https://doi.org/10.1017/S002531540000789X

Yonge, C. M. (1960). *Oysters*. Collins.

Young, S. R. (1979). Effect of temperature change on the metabolic rate of an antarctic mite. *Journal of Comparative Physiology*, *131*(4), 341–346. https://doi.org/10.1007/BF00688809

Yuan, Q., Metterville, D., Briscoe, A. D., & Reppert, S. M. (2007). Insect Cryptochromes: Gene Duplication and Loss Define Diverse Ways to Construct Insect Circadian Clocks. *Molecular Biology and Evolution*, *24*(4), 948–955. https://doi.org/10.1093/molbev/msm011

Yund, P. O. (2000). How severe is sperm limitation in natural populations of marine free-spawners? *Trends in Ecology & Evolution*, *15*(1), 10–13. https://doi.org/10.1016/S0169-5347(99)01744-9

Zantke, J., Ishikawa-Fujiwara, T., Arboleda, E., Lohs, C., Schipany, K., Hallay, N., Straw, A. D., Todo, T., & Tessmar-Raible, K. (2013). Circadian and Circalunar Clock Interactions in a Marine Annelid. *Cell Reports*, *5*(1), 99–113. https://doi.org/10.1016/j.celrep.2013.08.031

Zantke, J., Oberlerchner, H., & Tessmar-Raible, K. (2014). Circadian and Circalunar Clock Interactions and the Impact of Light in Platynereis dumerilii. In H. Numata & B. Helm (Eds.), *Annual, Lunar, and Tidal Clocks: Patterns and Mechanisms of Nature's Enigmatic Rhythms* (pp. 143–162). Springer Japan. https://doi.org/10.1007/978-4-431-55261-1_8

Zapata-Restrepo, L. M., Hauton, C., Williams, I. D., Jensen, A. C., & Hudson, M. D. (2019). Effects of the interaction between temperature and steroid hormones on gametogenesis and sex ratio in the European flat oyster (*Ostrea edulis)*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *236*, 110523. https://doi.org/10.1016/j.cbpa.2019.06.023

Zeng, Z., Ni, J., & Ke, C. (2013). Expression of glycogen synthase (*GYS*) and glycogen synthase kinase 3β (*GSK3*β) of the Fujian oyster, *Crassostrea angulata*, in relation to glycogen content in gonad development. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *166*(3), 203–214. https://doi.org/10.1016/j.cbpb.2013.09.003

Zhang, E. E., & Kay, S. A. (2010). Clocks not winding down: Unravelling circadian networks. *Nature Reviews Molecular Cell Biology*, *11*(11), 764–776. https://doi.org/10.1038/nrm2995

Zhang, L., Hastings, M. H., Green, E. W., Tauber, E., Sladek, M., Webster, S. G., Kyriacou, C. P., & Wilcockson, D. C. (2013). Dissociation of Circadian and Circatidal Timekeeping in the Marine Crustacean Eurydice pulchra. *Current Biology*, *23*(19), 1863–1873. https://doi.org/10.1016/j.cub.2013.08.038

Zhang, M., Kong, F., Ma, B., Chen, D., Ran, Z., Ma, S., Liao, K., Cao, J., Zhang, L., Yan, X., & Xu, J. (2023). Effects of light on growth, feeding rate, digestion, and antioxidation in juvenile razor clams *Sinonovacula constricta*. *Aquaculture*, *568*, 739306. https://doi.org/10.1016/j.aquaculture.2023.739306

Zhu, H., Yuan, Q., Froy, O., Casselman, A., & Reppert, S. M. (2005). The two CRYs of the butterfly. *Current Biology*, *15*(23), R953–R954. https://doi.org/10.1016/j.cub.2005.11.030

zu Ermgassen, P., Bos, O., Debney, A., Gamble, C., Glover, A., Pogoda, B., Pouvreau, S., Sanderson, W., Smith, D., Preston, J., Bromley, C., Ashton, E., Daly, P., Harris-Scott, E., Hausen, T., Hauton, C., Lown, A., Lynch, S., Tully, O., & Joyce, P. (2021). *European Native Oyster Habitat Restoration Monitoring Handbook*.

zu Ermgassen, P., Hancock, B., B, D., Greene, J., E, S., Spalding, M., & R., B. (2016). *Setting Objectives for Oyster Habitat Restoration Using Ecosystem Services: A Manager's Guide.*

zu Ermgassen, P., McCormick, H., Debney, A., Fariñas-Franco, J. M., Gamble, C., Gillies, C., Hancock, B., Laugen, A. T., Pouvreau, S., Preston, J., Sanderson, W. G., Strand, Å., & Thurstan, R. H. (2025). European Native Oyster Reef Ecosystems Are Universally Collapsed. *Conservation Letters*, *18*(1), e13068. https://doi.org/10.1111/conl.13068

zu Ermgassen, P. S. E., Thurstan, R. H., Corrales, J., Alleway, H., Carranza, A., Dankers, N., DeAngelis, B., Hancock, B., Kent, F., McLeod, I., Pogoda, B., Liu, Q., & Sanderson, W. G. (2020). The benefits of bivalve reef restoration: A global synthesis of underrepresented species. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *30*(11), 2050–2065. https://doi.org/10.1002/aqc.3410

Zurl, M., Poehn, B., Rieger, D., Krishnan, S., Rokvic, D., Veedin Rajan, V. B., Gerrard, E., Schlichting, M., Orel, L., Ćorić, A., Lucas, R. J., Wolf, E., Helfrich-Förster, C., Raible, F., & Tessmar-Raible, K. (2022). Two light sensors decode moonlight versus sunlight to adjust a plastic circadian/circalunidian clock to moon phase. *Proceedings of the National Academy of Sciences*, *119*(22), e2115725119. https://doi.org/10.1073/pnas.2115725119

Zwerschke, N., Kochmann, J., Ashton, E., Crowe, T., Roberts, D., & O'Connor, N. (2017). Co-occurrence of native Ostrea edulis and non-native Crassostrea gigas revealed by monitoring of intertidal oyster populations. *Journal of the Marine Biological Association of the United Kingdom*, *98*, 1–10. https://doi.org/10.1017/S0025315417001448

9. Appendix

9.1 Supplementary material for Article I

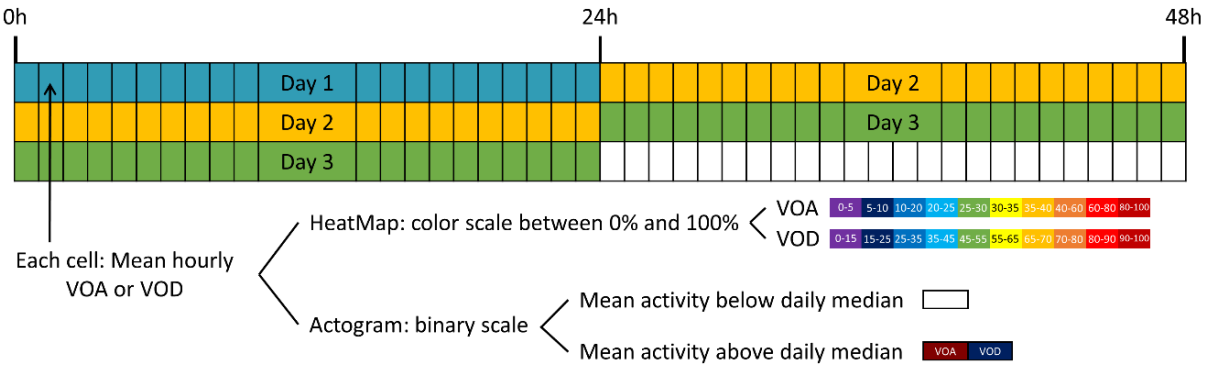
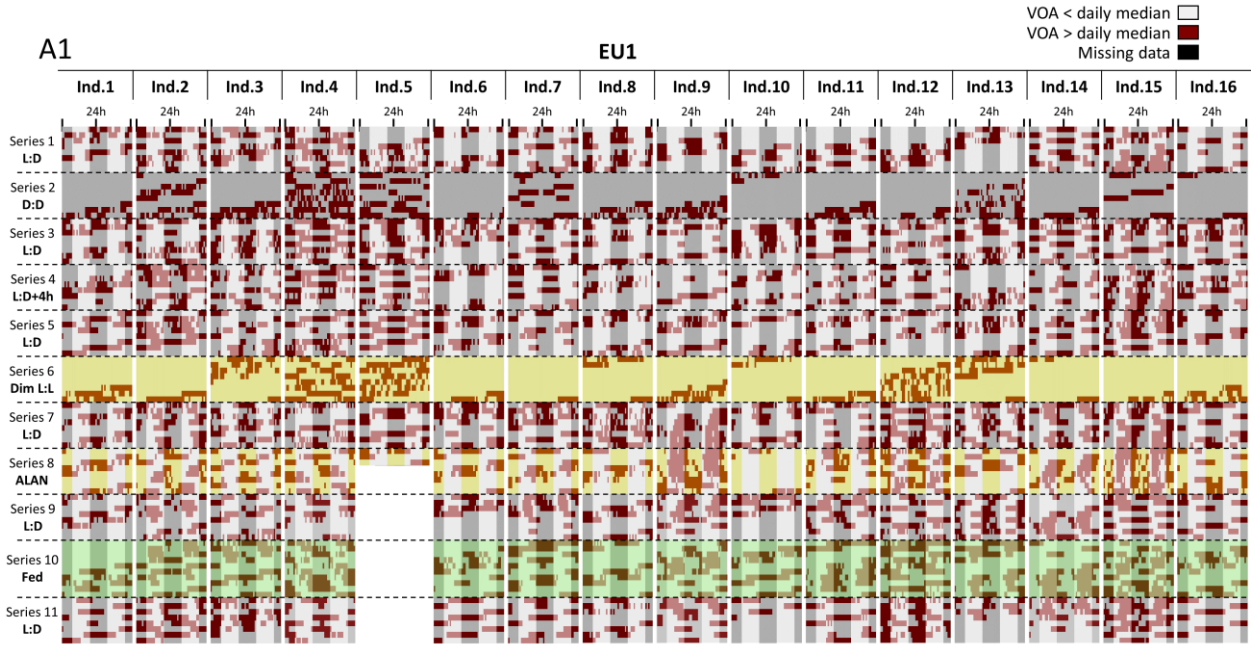
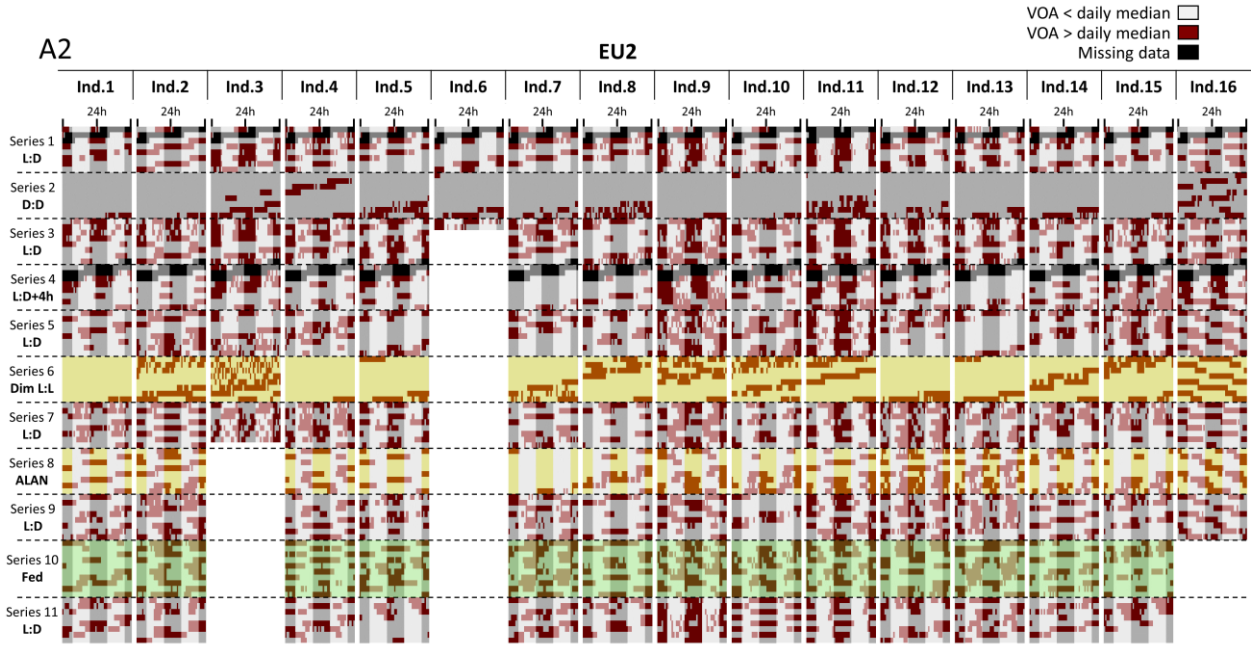


Figure S9.1. Schematic principle of actogram and heatmap representations of mean valve opening amplitude (VOA) and mean valve opening duration (VOD).

Table S9.1. Individual chronobiological analyses of valve activity. Results of the individual chronobiological analysis for VOA and VOD for the two experimental units (EU1 and EU2). For each individual during each series is associated the characteristics of the Cosinor model: the period (h) ± SE and the *p-value*. "ns" represent a non-significant Cosinor model. Dark grey cells represent dead animals (or signal problem during series 1 for the EU2). The last line shows the mean individual period ± SE for each series.

		VOA											VOD										
	Series	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	10	11
EU 1	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	26.1 ± 0.07 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	26.3 ± 0.07 p<0.001
	2	27.2 ± 0.08 p<0.001	ns	24.4 ± 0.07 p<0.001	23.6 ± 0.09 p<0.001	21.6 ± 0.09 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	24.4 ± 0.09 p<0.001	ns	ns	27.6 ± 0.06 p<0.001
	3	23.8 ± 0.08 p<0.001	ns	23.9 ± 0.08 p<0.001	26.9 ± 0.05 p<0.001	25.3 ± 0.08 p<0.001	ns	23.2 ± 0.07 p<0.001	22.5 ± 0.08 p<0.001	24.9 ± 0.06 p<0.001	ns	ns	ns	ns	ns	25.8 ± 0.06 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns
	4	ns	26.0 ± 0.08 p<0.001	ns	ns	ns	ns	ns	27.4 ± 0.05 p<0.001	25.2 ± 0.06 p<0.001	ns	25.8 ± 0.05 p<0.001	ns	26.6 ± 0.07 p<0.001	ns	ns	ns	ns	ns	27.6 ± 0.07 p<0.001	25.5 ± 0.07 p<0.001	22.0 ± 0.09 p<0.001	25.7 ± 0.06 p<0.001
	5	ns	ns	24.8 ± 0.05 p<0.001	ns	ns	ns	ns	Dead				ns	ns	24.3 ± 0.08 p<0.001	ns	ns	ns	ns	Dead				
	6	23.0 ± 0.10 p<0.001	ns	ns	23.6 ± 0.09 p<0.001	22.1 ± 0.07 p<0.001	ns	ns	ns	ns	22.3 ± 0.12 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	7	ns	24.3 ± 0.07 p<0.001	ns	22.8 ± 0.08 p<0.001	22.4 ± 0.09 p<0.001	ns	ns	ns	23.7 ± 0.09 p<0.001	23.8 ± 0.08 p<0.001	23.5 ± 0.07 p<0.001	ns	ns	ns	22.6 ± 0.09 p<0.001	ns	ns	ns	ns	ns	ns	23.8 ± 0.08 p<0.001
	8	24.0 ± 0.05 p<0.001	ns	23.6 ± 0.05 p<0.001	ns	ns	ns	ns	27.4 ± 0.06 p<0.001	ns	ns	ns	23.5 ± 0.09 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	9	26.4 ± 0.06 p<0.001	ns	ns	ns	ns	ns	23.9 ± 0.06 p<0.001	22.9 ± 0.06 p<0.001	23.6 ± 0.06 p<0.001	20.9 ± 0.09 p<0.001	26.1 ± 0.05 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	23.4 ± 0.07 p<0.001	ns	25.2 ± 0.07 p<0.001
	10	22.1 ± 0.09 p<0.001	ns	26.2 ± 0.05 p<0.001	ns	ns	ns	24.0 ± 0.07 p<0.001	ns	ns	ns	27.6 ± 0.06 p<0.001	21.8 ± 0.11 p<0.001	ns	26.9 ± 0.07 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	28.0 ± 0.07 p<0.001
	11	27.0 ± 0.08 p<0.001	ns	ns	ns	ns	ns	25.6 ± 0.07 p<0.001	25.3 ± 0.05 p<0.001	ns	23.7 ± 0.06 p<0.001	ns	ns	ns	ns	24.3 ± 0.09 p<0.001	ns	ns	25.2 ± 0.06 p<0.001	ns	ns	ns	ns
	12	25.1 ± 0.08 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	24.1 ± 0.05 p<0.001	ns	ns	25.7 ± 0.08 p<0.001	22.0 ± 0.10 p<0.001	ns	ns	ns	ns	ns	ns	24.2 ± 0.08 p<0.001
	13	ns	ns	ns	ns	ns	ns	23.5 ± 0.08 p<0.001	ns	22.9 ± 0.07 p<0.001	23.6 ± 0.09 p<0.001	22.5 ± 0.09 p<0.001	ns	ns	ns	ns	ns	ns	26.2 ± 0.08 p<0.001	26.9 ± 0.07 p<0.001	23.7 ± 0.06 p<0.001	23.6 ± 0.11 p<0.001	ns
	14	ns	ns	20.0 ± 0.12 p<0.001	23.7 ± 0.06 p<0.001	24.2 ± 0.08 p<0.001	ns	24.6 ± 0.05 p<0.001	22.8 ± 0.07 p<0.001	23.0 ± 0.07 p<0.001	24.0 ± 0.06 p<0.001	25.1 ± 0.08 p<0.001	ns	ns	ns	ns	23.9 ± 0.07 p<0.001	ns	25.0 ± 0.05 p<0.001	ns	ns	ns	ns
	15	ns	ns	ns	24.2 ± 0.05 p<0.001	24.6 ± 0.06 p<0.001	ns	22.4 ± 0.05 p<0.001	23.4 ± 0.08 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	16	ns	ns	ns	ns	22.6 ± 0.07 p<0.001	ns	ns	25.5 ± 0.05 p<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
EU 2	1	Signal problem	ns	22.6 ± 0.09 p<0.001	22.6 ± 0.07 p<0.001	ns	ns	24.0 ± 0.08 p<0.001	ns	ns	ns	ns	Signal problem	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	2		ns	27.2 ± 0.08 p<0.001	24.4 ± 0.05 p<0.001	25.4 ± 0.06 p<0.001	ns	ns	27.7 ± 0.06 p<0.001	25.6 ± 0.04 p<0.001	24.2 ± 0.07 p<0.001	23.6 ± 0.09 p<0.001		ns	ns	24.4 ± 0.05 p<0.001	ns	ns	ns	ns	25.6 ± 0.07 p<0.001	ns	ns
	3		ns	24.1 ± 0.07 p<0.001	22.5 ± 0.08 p<0.001	ns	ns	ns	Dead					ns	ns	ns	ns	ns	25.2 ± 0.07 p<0.001	Dead			
	4		ns	ns	24.3 ± 0.05 p<0.001	ns	ns	25.1 ± 0.08 p<0.001	ns	26.1 ± 0.07 p<0.001	23.4 ± 0.09 p<0.001	ns		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	5		ns	24.2 ± 0.05 p<0.001	24.1 ± 0.06 p<0.001	24.0 ± 0.09 p<0.001	ns	23.6 ± 0.08 p<0.001	ns	24.8 ± 0.07 p<0.001	ns	25.5 ± 0.05 p<0.001		ns	24.2 ± 0.05 p<0.001	23.7 ± 0.05 p<0.001	ns	ns	ns	ns	ns	24.1 ± 0.09 p<0.001	ns
	6		ns	Dead										ns	Dead								
	7		ns	ns	ns	ns	ns	ns	ns	25.7 ± 0.08 p<0.001	24.3 ± 0.08 p<0.001	ns		ns	ns	ns	ns	ns	ns	27.4 ± 0.06 p<0.001	ns	ns	ns
	8		ns	ns	ns	22.6 ± 0.08 p<0.001	ns	24.0 ± 0.07 p<0.001	ns	ns	24.6 ± 0.10 p<0.001	26.3 ± 0.07 p<0.001		ns	ns	ns	ns	ns	23.9 ± 0.08 p<0.001	ns	ns	ns	ns
	9		ns	23.0 ± 0.08 p<0.001	ns	ns	ns	21.7 ± 0.09 p<0.001	27.2 ± 0.03 p<0.001	21.0 ± 0.11 p<0.001	ns	24.2 ± 0.04 p<0.001		ns	ns	ns	ns	ns	ns	ns	25.5 ± 0.08 p<0.001	23.6 ± 0.08 p<0.001
	10		ns	ns	ns	23.4 ± 0.09 p<0.001	ns	25.1 ± 0.06 p<0.001	24.5 ± 0.07 p<0.001	23.6 ± 0.09 p<0.001	23.6 ± 0.09 p<0.001	ns		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	11		ns	24.3 ± 0.04 p<0.001	ns	24.1 ± 0.05 p<0.001	ns	25.5 ± 0.04 p<0.001	25.1 ± 0.09 p<0.001	22.9 ± 0.06 p<0.001	23.5 ± 0.07 p<0.001	24.2 ± 0.04 p<0.001		ns	24.2 ± 0.07 p<0.001	ns	20.1 ± 0.09 p<0.001	ns	25.6 ± 0.06 p<0.001	ns	27.4 ± 0.05 p<0.001	ns	24.3 ± 0.08 p<0.001
	12		ns	24.2 ± 0.06 p<0.001	ns	23.1 ± 0.07 p<0.001	ns	24.0 ± 0.06 p<0.001	24.1 ± 0.07 p<0.001	24.2 ± 0.07 p<0.001	27.6 ± 0.05 p<0.001	23.0 ± 0.08 p<0.001		ns	ns	25.6 ± 0.06 p<0.001	ns	ns	23.6 ± 0.07 p<0.001	ns	ns	21.6 ± 0.12 p<0.001	23.4 ± 0.10 p<0.001
	13		ns	24.4 ± 0.05 p<0.001	ns	ns	ns	24.8 ± 0.07 p<0.001	ns	ns	21.7 ± 0.08 p<0.001	ns		ns	ns	ns	ns	ns	ns	ns	ns	22.1 ± 0.11 p<0.001	ns
	14		ns	20.8 ± 0.11 p<0.001	24.4 ± 0.08 p<0.001	25.7 ± 0.08 p<0.001	ns	25.4 ± 0.06 p<0.001	ns	ns	ns	ns		ns	24.8 ± 0.08 p<0.001	ns	ns	ns	25.1 ± 0.08 p<0.001	ns	ns	ns	ns
	15		ns	ns	22.3 ± 0.05 p<0.001	24.5 ± 0.06 p<0.001	ns	25.2 ± 0.07 p<0.001	22.6 ± 0.08 p<0.001	23.8 ± 0.09 0.0001	ns	26.9 ± 0.06 p<0.001		ns	ns	ns	ns	ns	ns	ns	ns	ns	25.8 ± 0.07 p<0.001
	16		ns	ns	23.8 ± 0.06 p<0.001	23.7 ± 0.07 p<0.001	ns	ns	23.7 ± 0.08 p<0.001	ns	Dead			ns	ns	24.6 ± 0.07 p<0.001	23.6 ± 0.08 p<0.001	ns	ns	23.6 ± 0.07 p<0.001	ns	Dead	
	Mean	24.5 ± 0.87	25.1 ± 0.85	23.8 ± 0.46	23.8 ± 0.31	23.7 ± 0.31	/	24.4 ± 0.32	24.8 ± 0.49	24.1 ± 0.35	23.6 ± 0.41	24.9 ± 0.39	22.6 ± 0.85	26.6 ± 0.00	25.0 ± 0.44	24.1 ± 0.47	22.5 ± 1.22	/	25.0 ± 0.30	26.0 ± 0.83	25.1 ± 0.73	23.1 ± 0.62	25.3 ± 0.78


A1
EU1
VOA < daily median
VOA > daily median
Missing data
Ind.1
Ind.2
Ind.3
Ind.4
Ind.5
Ind.6
Ind.7
Ind.8
Ind.9
Ind.10
Ind.11
Ind.12
Ind.13
Ind.14
Ind.15
Ind.16
24h
Series 1 L:D
Series 2 D:D
Series 3 L:D
Series 4 L:D+4h
Series 5 L:D
Series 6 Dim L:L
Series 7 L:D
Series 8 ALAN
Series 9 L:D
Series 10 Fed
Series 11 L:D


A2
EU2
VOA < daily median
VOA > daily median
Missing data
Ind.1
Ind.2
Ind.3
Ind.4
Ind.5
Ind.6
Ind.7
Ind.8
Ind.9
Ind.10
Ind.11
Ind.12
Ind.13
Ind.14
Ind.15
Ind.16
24h
Series 1 L:D
Series 2 D:D
Series 3 L:D
Series 4 L:D+4h
Series 5 L:D
Series 6 Dim L:L
Series 7 L:D
Series 8 ALAN
Series 9 L:D
Series 10 Fed
Series 11 L:D

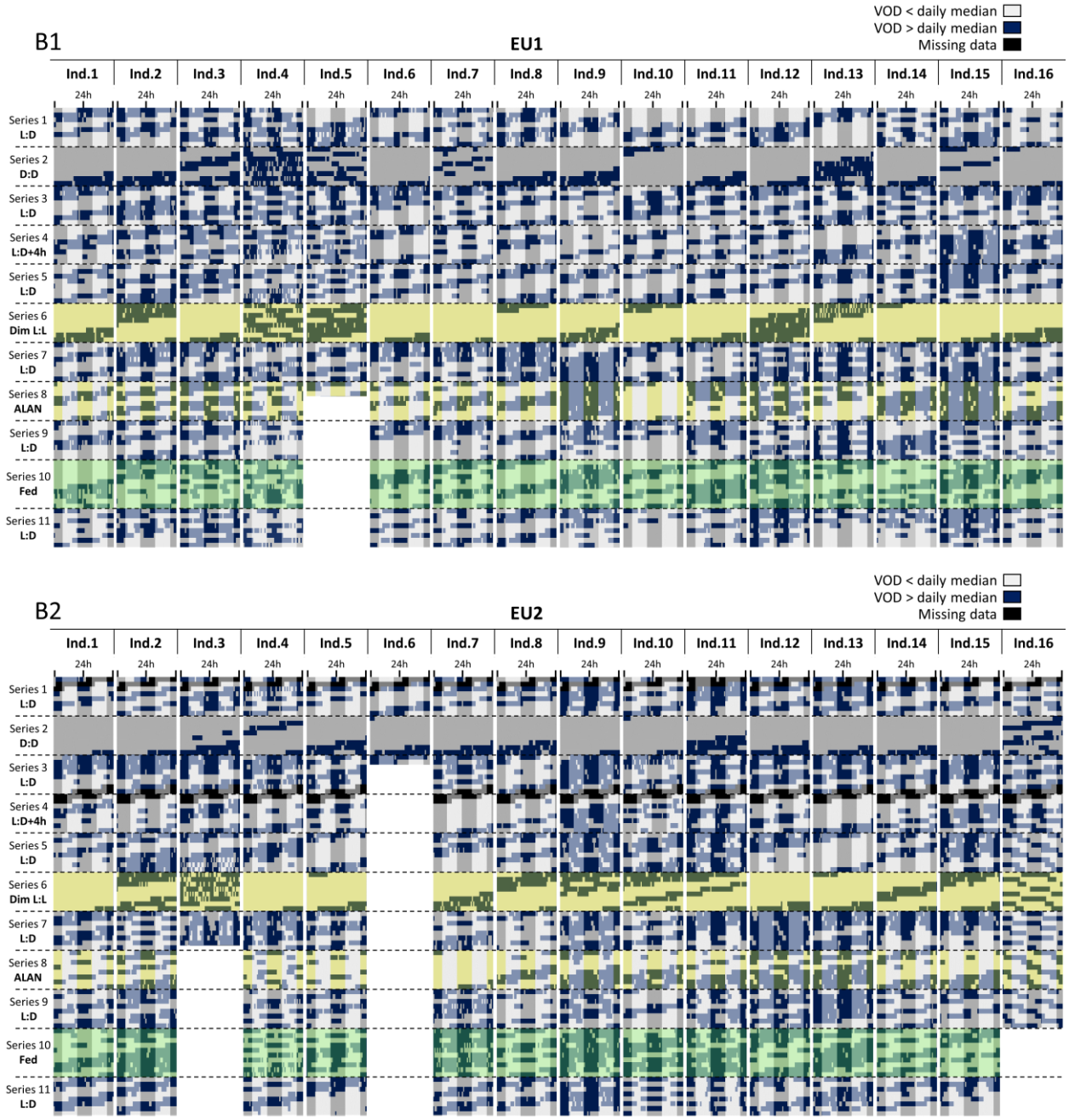
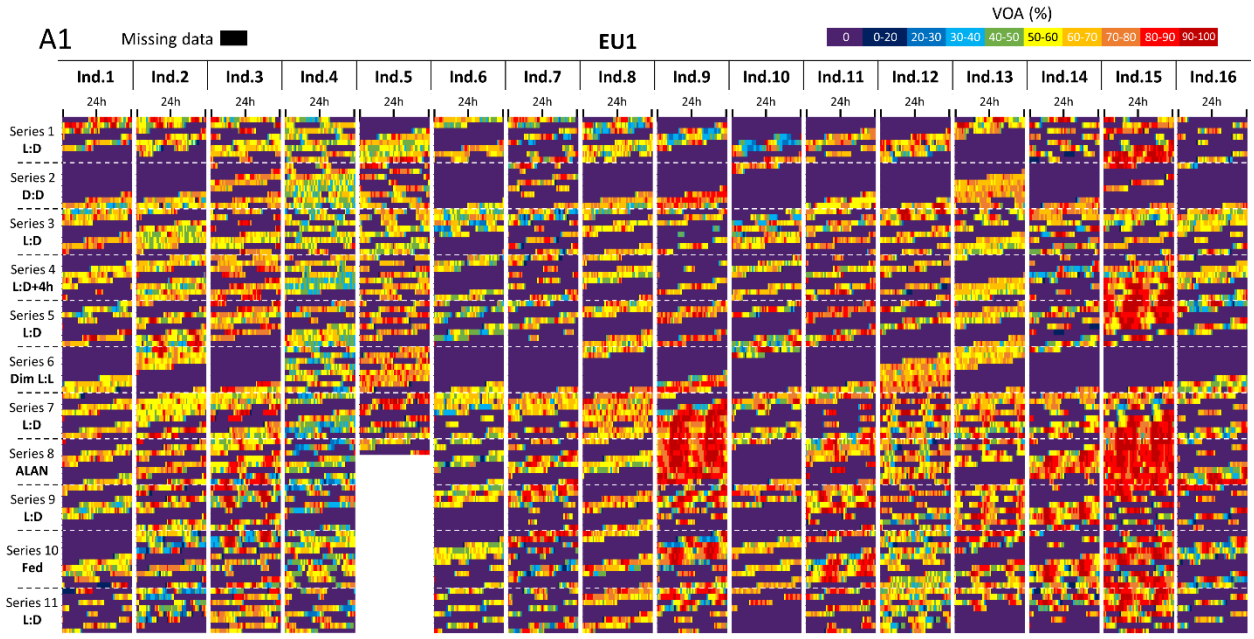
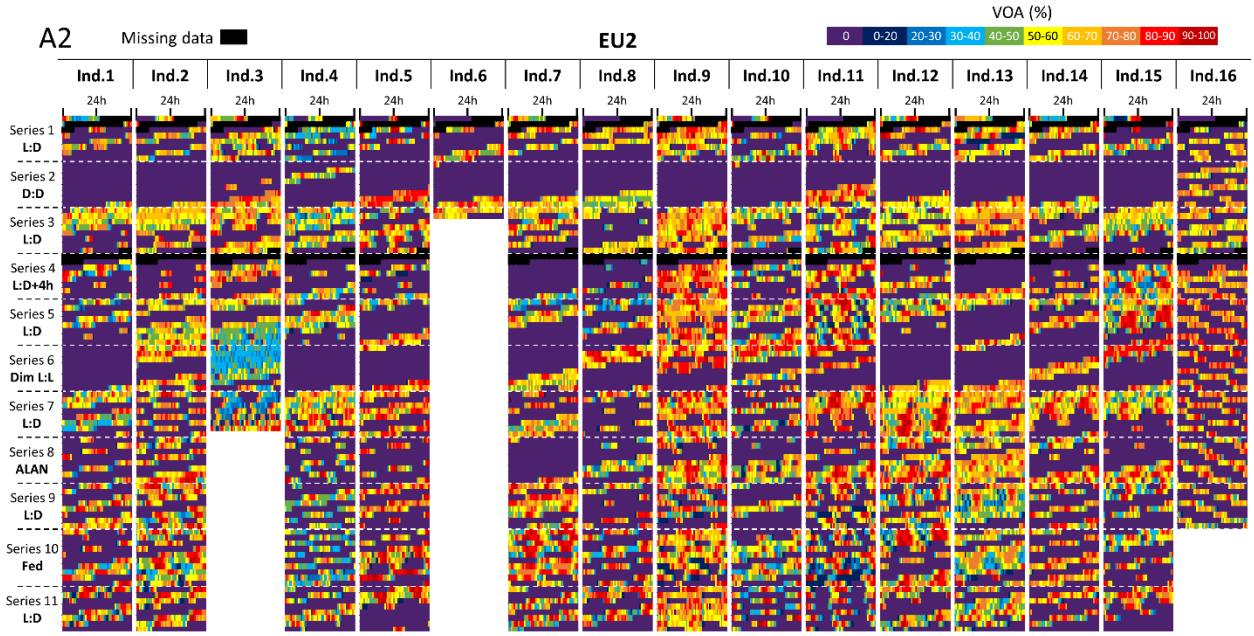


Figure S9.2. Actograms of *Ostrea edulis* valve activity at the individual level. Actograms of VOA (A1-A2) and VOD (B1-B2) during the 11 series, 97 days in total for the 32 individuals divided in 2 experimental units (EU1 and EU2). On actograms, dark grey areas = scotophase, white areas = photophase, yellow area = dim light exposure, green area = continuous food supply. The 11 series are detailed in Fig. 1B.


A1
Missing data
EU1
VOA (%)
0
0-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
Ind.1
Ind.2
Ind.3
Ind.4
Ind.5
Ind.6
Ind.7
Ind.8
Ind.9
Ind.10
Ind.11
Ind.12
Ind.13
Ind.14
Ind.15
Ind.16
24h
Series 1 L:D
Series 2 D:D
Series 3 L:D
Series 4 L:D+4h
Series 5 L:D
Series 6 Dim L:L
Series 7 L:D
Series 8 ALAN
Series 9 L:D
Series 10 Fed
Series 11 L:D


A2
Missing data
EU2
VOA (%)
0
0-20
20-30
30-40
40-50
50-60
60-70
70-80
80-90
90-100
Ind.1
Ind.2
Ind.3
Ind.4
Ind.5
Ind.6
Ind.7
Ind.8
Ind.9
Ind.10
Ind.11
Ind.12
Ind.13
Ind.14
Ind.15
Ind.16
24h
Series 1 L:D
Series 2 D:D
Series 3 L:D
Series 4 L:D+4h
Series 5 L:D
Series 6 Dim L:L
Series 7 L:D
Series 8 ALAN
Series 9 L:D
Series 10 Fed
Series 11 L:D

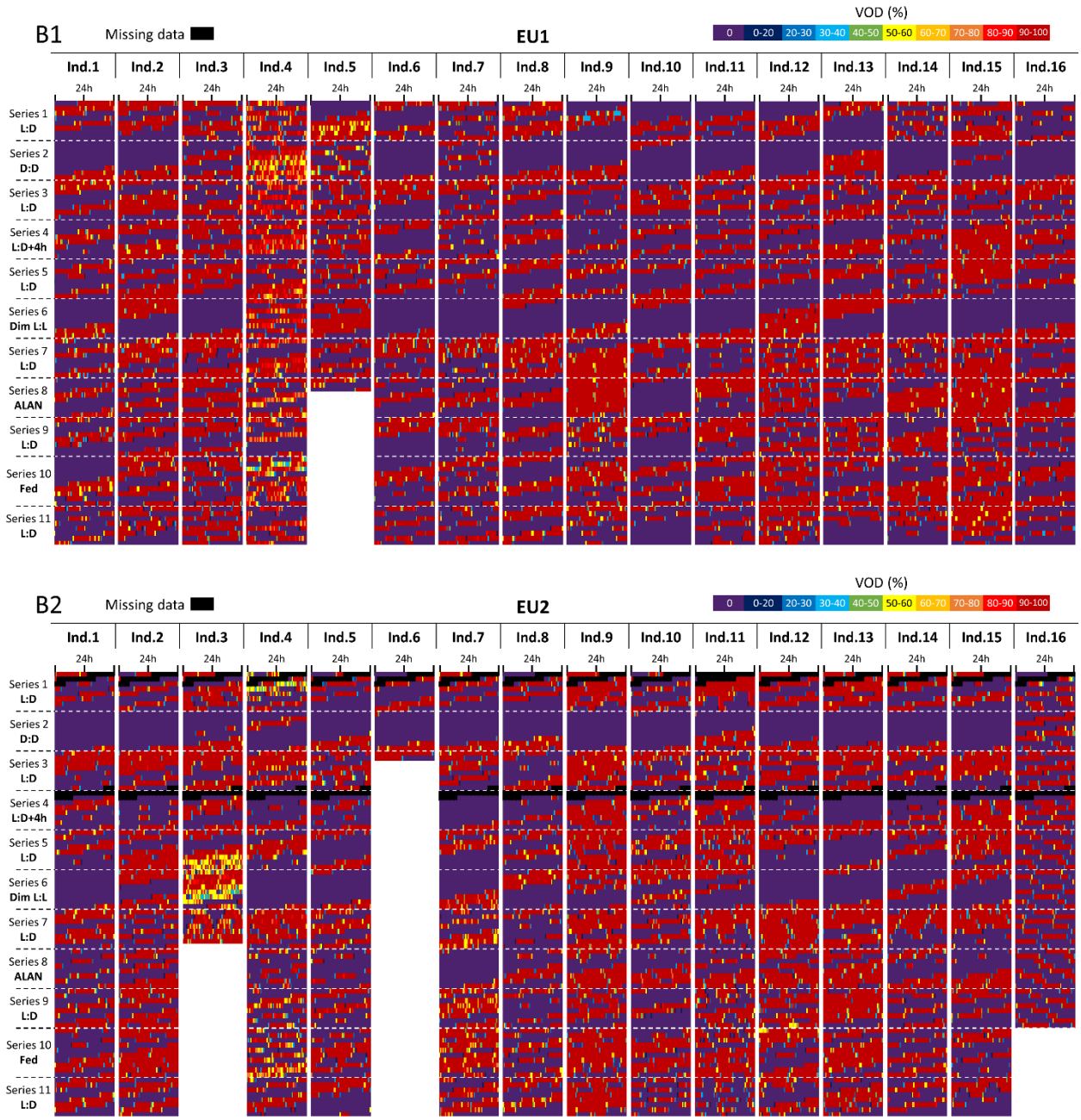


Figure S9.3. Daily pattern of *Ostrea edulis* valve behavior at the individual level. Heatmaps of VOA (A1-A2) and VOD (B1-B2) during the 11 series, 97 days in total for the 32 individuals divided in 2 experimental units (EU1 and EU2). The 11 series are detailed in Fig. 1B.

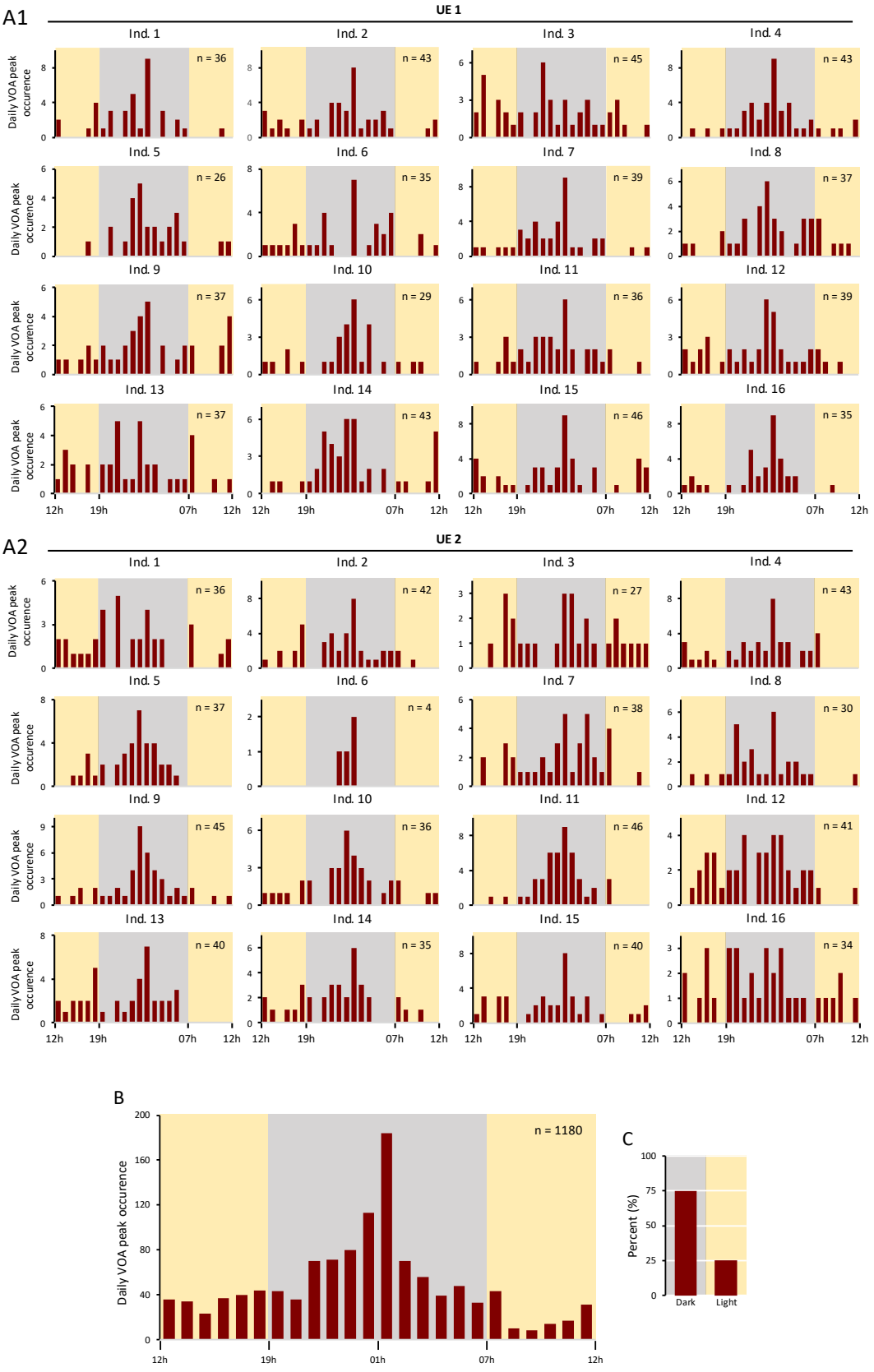

A1
UE 1
Ind. 1
n = 36
Ind. 2
n = 43
Ind. 3
n = 45
Ind. 4
n = 43
Ind. 5
n = 26
Ind. 6
n = 35
Ind. 7
n = 39
Ind. 8
n = 37
Ind. 9
n = 37
Ind. 10
n = 29
Ind. 11
n = 36
Ind. 12
n = 39
Ind. 13
n = 37
Ind. 14
n = 43
Ind. 15
n = 46
Ind. 16
n = 35
Daily VOA peak occurence
12h
19h
07h
12h
A2
UE 2
Ind. 1
n = 36
Ind. 2
n = 42
Ind. 3
n = 27
Ind. 4
n = 43
Ind. 5
n = 37
Ind. 6
n = 4
Ind. 7
n = 38
Ind. 8
n = 30
Ind. 9
n = 45
Ind. 10
n = 36
Ind. 11
n = 46
Ind. 12
n = 41
Ind. 13
n = 40
Ind. 14
n = 35
Ind. 15
n = 40
Ind. 16
n = 34
Daily VOA peak occurence
12h
19h
07h
12h
B
n = 1180
Daily VOA peak occurence
12h
19h
01h
07h
12h
C
Percent (%)
Dark
Light

Figure S9.4. Daily valve activity peak of *Ostrea edulis* under L:D regimes at individual level. (A1-A2) Histograms of daily VOA peak occurrences during L:D regimes (series 1, 3, 5, 7, 9, and 11) for the 32 individuals divided in 2 experimental units (EU1 and EU2). (B) Histograms of daily VOA peak occurrences during L:D regimes for all the individuals (n= 32). (C) Percentage of daily VOA peaks during scotophase and photophase for all individuals (n = 32) during all L:D series. Dark grey areas represent scotophase, yellow areas represent photophase. "n" in each graph indicates the number of days taken into account, i.e. the number of daily peaks. Days of continuous closure are excluded.

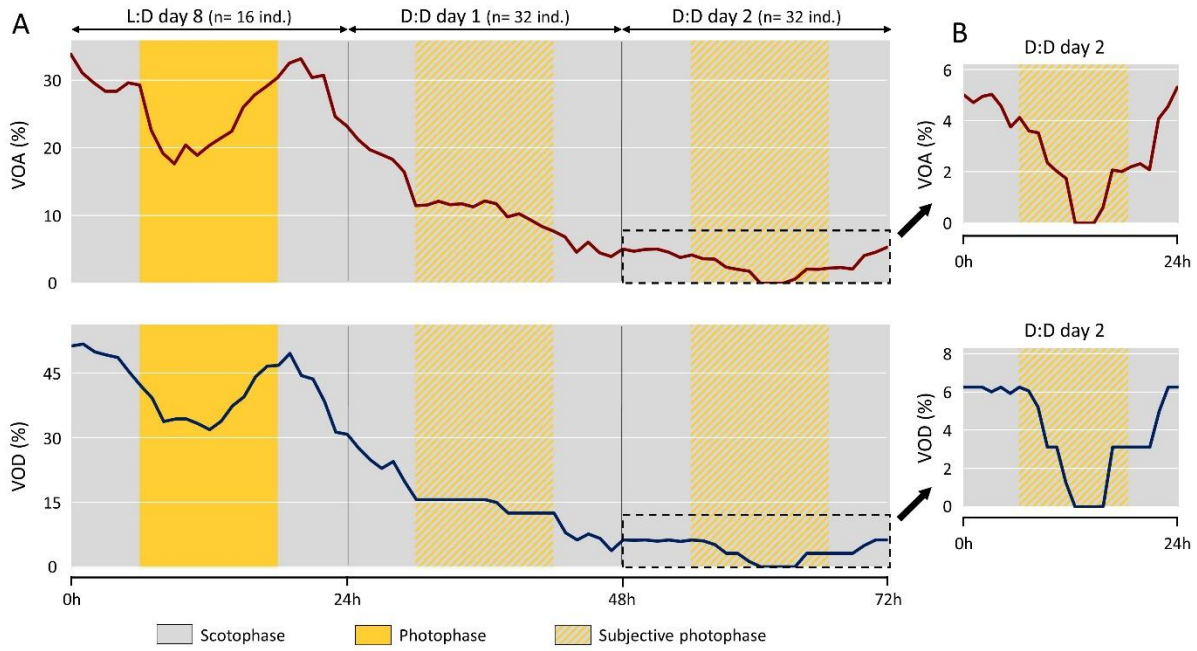


Figure S9.5. Transition of *Ostrea edulis* valve activity from L:D to D:D. (A) Daily profile of mean VOA (red line) and mean VOD (blue line) during the last day of L:D (Series 1) and the two first days of D:D (Series 2) (B) Zoom on the mean VOA and VOD profiles during the second day of D:D. Grey areas represent scotophase, yellow areas represent photophase, yellow hatched areas represent subjective photophase. n = 16-32.

9.2 Supplementary material for Article II

Table S9.2. Accession numbers of identified genes of interest in *Ostrea edulis*. Abbreviations (Abbrev.) and accession numbers for house-keeping genes used for the molecular analysis are in blue.

Designation	Abbrev.	Size (aa)	Accession Number (NCBI)	Scaffold	Start - End	Forward sequence for primer design	Reverse sequence for primer design
Core circadian clock genes							
OeClock	*OeClock*	677	XM_048900754.1	NC_079165.1	105.106.218 – 105.168.468	CAGCCACCAGTA CCCAGAAT	TCTGGCTCGGA GATTTCTGT
OeBmal1	*OeBmal1*	546	XM_048874853.1	NC_079169.1	26.404.696 - 26.503.908	ACGAGAGGGTGC AGCTATGT	AGGATGTTCCG CACTGATTC
OePeriod	*OePer1*	1314	XM_048874757.1	NC_079164.1	63.547.232 - 63.588.033	GGCACATGCTGA ACAAGAGA	AGAGGAAATGG TGGCACAAG
OeTimeless	*OeTim1*	949	XM_048891525.1	NC_079172.1	63.985.766 - 64.023.320	AGGAACCAGTGG CACGATAC	TGGGAAACACA AGACCAACA
OeCryptochrom e2	*OeCry2*	1255	XM_048913721.1	NC_079166.1	64.451.980 – 64.515.807	CGAGGTCAGCAA ACTCAACA	TACTCGTGAGG GATGGTTCC
Clock-related genes							
OeRev-erb	*OeRev-erb*	1089	XM_048918733.1	NC_079165.1	49.319.823 - 49.451.100	CTGTTCCGCCAC AATTCTTT	CCTTGGACGCA ATTGAGAAT
OeRor	*OeRor*	502	XM_048910521.1	NC_079166.1	94.459.429 - 94.465.999	CTCCCCAAGTCA CCTCTCAG	GAGGGGTCATG CTGGATTTA
OeDoubletime	*OeDbt*	426	XM_048904376.1	NC_079167.1	81.880.562 - 81.898.047	GAATCGCTGGGA TACGTGTT	CAGGGATCGGC AAAAGTTTA
OeClockWork Orange	*OeCwo*	376	XM_048885347.1	NC_079170.1	34.299.045 - 34.314.356	GCCCAAAGAACA TTCAAGGA	GGTGCTTCTTC TGGAGCTTG
OeShaggy	*OeShag*	412	XM_048923210.1	NC_079165.1	94.700.493 - 94.716.280	AGATCCAAACAC CAGGTTGC	GATTGGTGTCT CCCGACAGT
Gene involved in melatonin synthesis							
OeHiomt	*OeHiomt*	410	XM_048878998.1	NC_079168.1	91.258.088 - 91.273.886	TTGCAGAAGGAT TGCAACAG	ATGATCATGGC ATCGACAGA
Photoreceptor genes							
OeCryptochrom e1	*OeCry1*	545	XM_048872495.1	NC_079169.1	28.616.332 - 28.647.748	GTCTCGCACACT CTGTGGAA	GTCCGTGAAGT CTGGGTCAT
OeOpsin4	*OeOpn4*	384	XM_048908333.1	NC_079167.1	64.571.865 - 64.581.864	GACGAGGCTGGA CAGAAAAC	GACGGTCCTGG TTGAGTGTT
Housekeeping genes							
OeGAPDH	*OeGapdh*	1317	XM_048908150.1	NC_079167.1	32.697.835 - 32.699.467	TGCAATCAATGA TCCCTTCA	AGCCTTTCCGC TGATCACTA
OeEF1	*OeEf1*	1755	XM_048873495.1	NC_079169.1	17.275.318 - 17.288.795	CCAGGCCACAGA GATTTCAT	TTGACTCCGAC AATGAGCTG
Oe28S	*Oe28S*	640	XM_048873299.1	NC_079169.1	32.819.931 - 32.823.362	TGGGGAAAGTGA TAGACGTTG	AACCTCCTCCT TCCCAGTGT

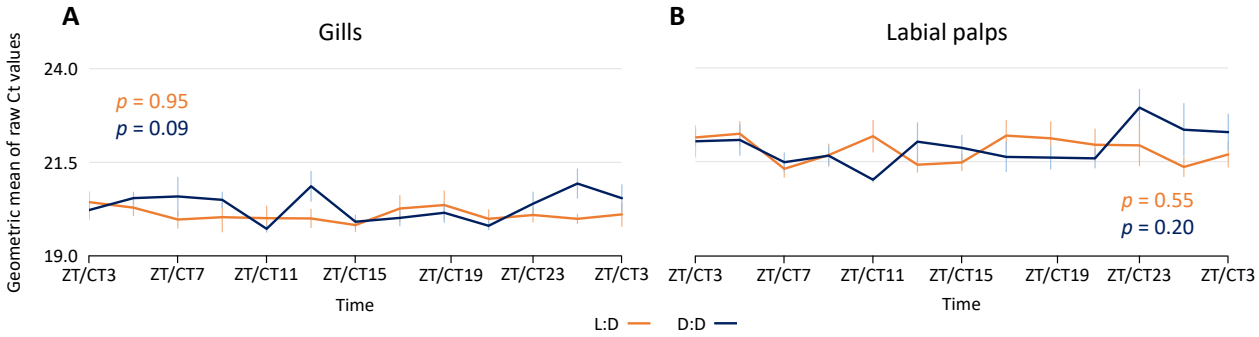
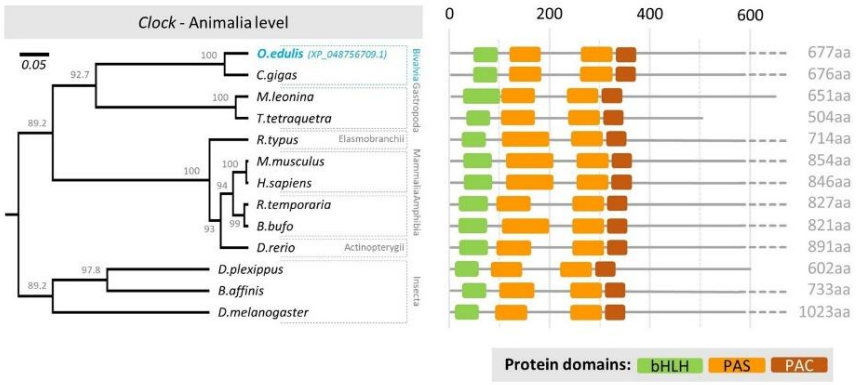
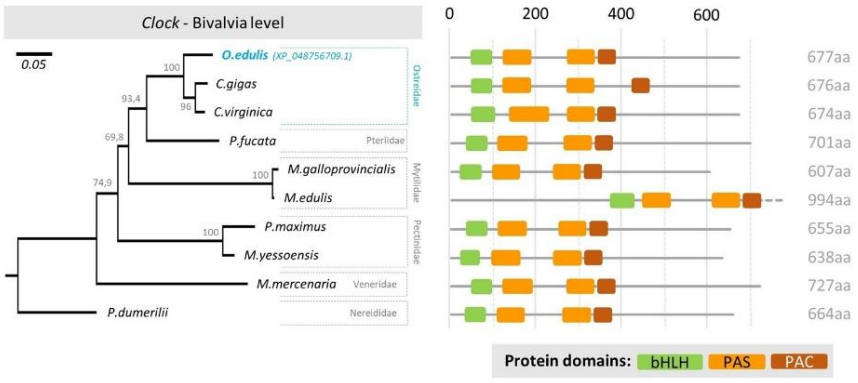


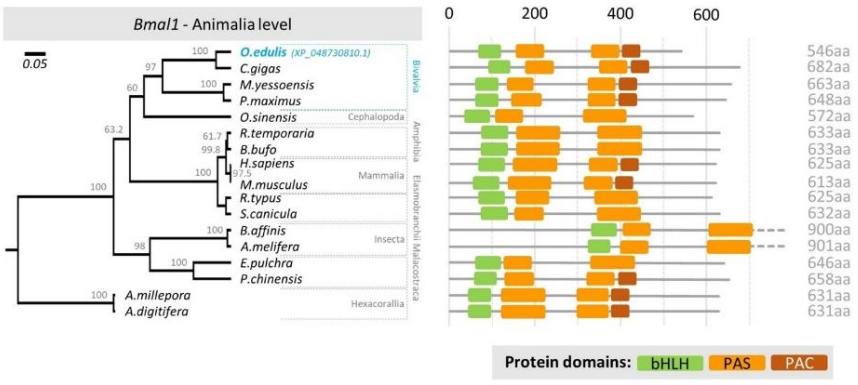
Figure S9.6. Temporal stability of the housekeeping genes (*OeEf1*, *OeGapdh*, and *Oe28S*). Geometric mean ± standard deviation of raw Ct values from three housekeeping genes (*OeEf1*, *OeGapdh*, and *Oe28S*) under light:dark (LD, orange line) and constant darkness (DD, blue line) conditions in (A) gills and (B) labial palps. *p*-values shown on the figures correspond to the results of one-way ANOVA testing for differences between time points, with significance considered at $p < 0.05$. ZT: *zeitgeber* time; CT: circadian time.

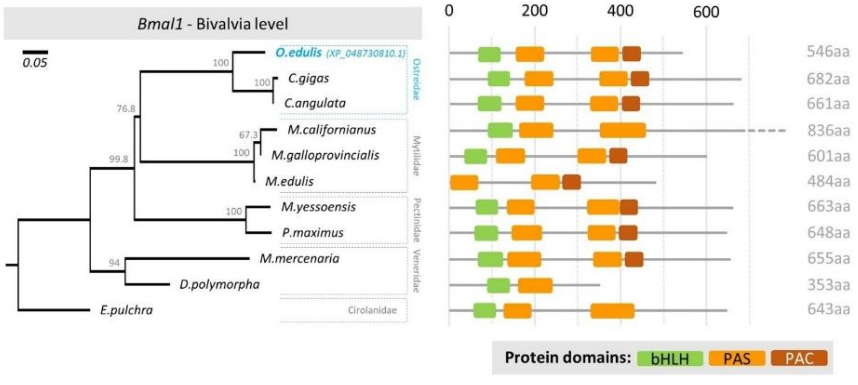
Clock


Clock - Animalia level
0.05
O.edulis (XP_048756709.1)
C.gigas
M.leonina
T.tetraquetra
R.typus
M.musculus
H.sapiens
R.temporaria
B.bufo
D.rerio
D.plexippus
B.affinis
D.melanogaster
Bivalvia
Gastropoda
Elasmobranchii
Mammalia
Amphibia
Actinopterygii
Insecta
0
200
400
600
677aa
676aa
651aa
504aa
714aa
854aa
846aa
827aa
821aa
891aa
602aa
733aa
1023aa
Protein domains: bHLH PAS PAC

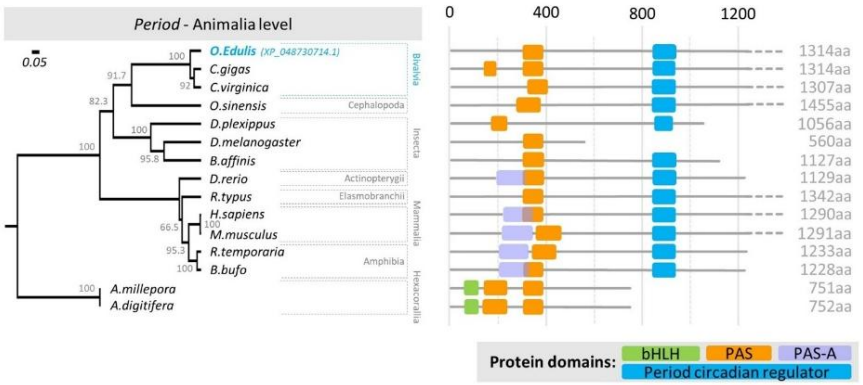

Clock - Bivalvia level
0.05
O.edulis (XP_048756709.1)
C.gigas
C.virginica
P.fucata
M.galloprovincialis
M.edulis
P.maximus
M.yessoensis
M.mercenaria
P.dumerilii
Ostreidae
Pteriidae
Mytilidae
Pectinidae
Veneridae
Nereididae
0
200
400
600
677aa
676aa
674aa
701aa
607aa
994aa
655aa
638aa
727aa
664aa
Protein domains: bHLH PAS PAC

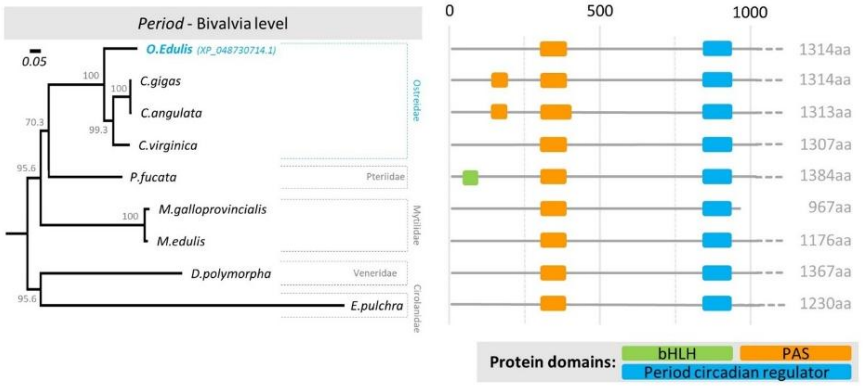
Bmal1


Bmal1 - Animalia level
0.05
O.edulis (XP_048730810.1)
C.gigas
M.yessoensis
P.maximus
O.sinensis
R.temporaria
B.bufo
H.sapiens
M.musculus
R.typus
S.canicula
B.affinis
A.melifera
E.pulchra
P.chinensis
A.millepora
A.digitifera
Bivalvia
Cephalopoda
Amphibia
Mammalia
Elasmobranchii
Malacostraca
Insecta
Hexacorallia
0
200
400
600
546aa
682aa
663aa
648aa
572aa
633aa
633aa
625aa
613aa
625aa
632aa
900aa
901aa
646aa
658aa
631aa
631aa
Protein domains: bHLH PAS PAC

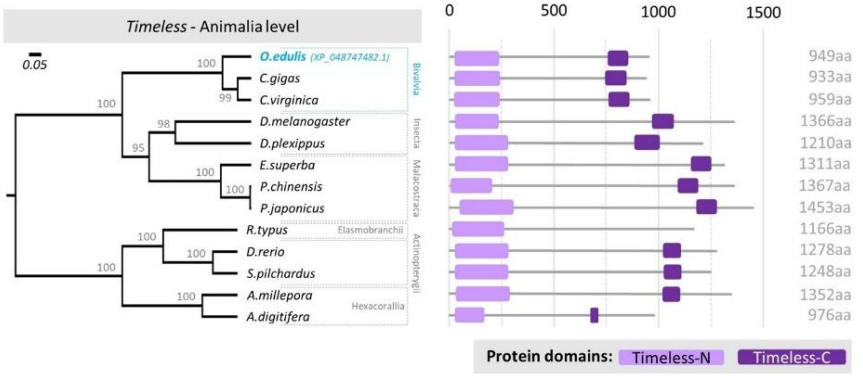

Bmal1 - Bivalvia level
0.05
O.edulis (XP_048730810.1)
C.gigas
C.angulata
M.californianus
M.galloprovincialis
M.edulis
M.yessoensis
P.maximus
M.mercenaria
D.polymorpha
E.pulchra
Ostreidae
Mytilidae
Pectinidae
Veneridae
Cirolanidae
0
200
400
600
546aa
682aa
661aa
836aa
601aa
484aa
663aa
648aa
655aa
353aa
643aa
Protein domains: bHLH PAS PAC

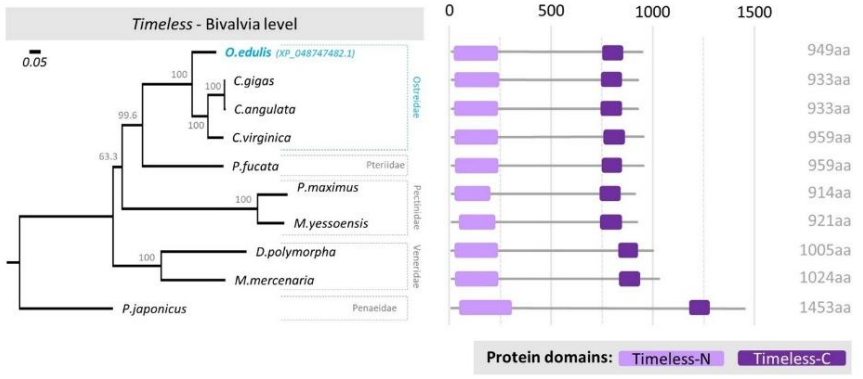
Period



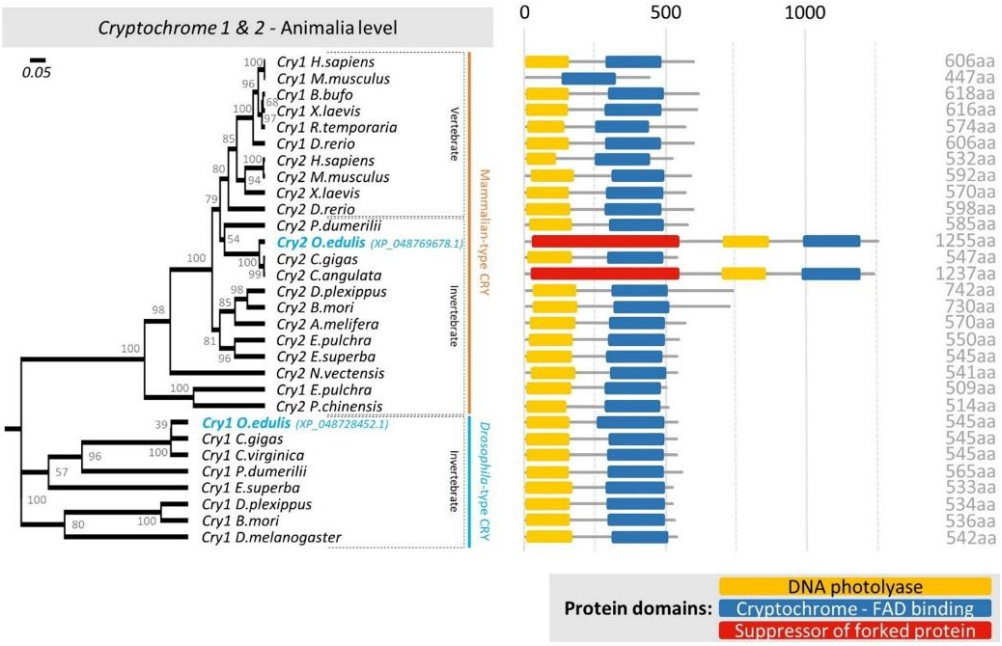


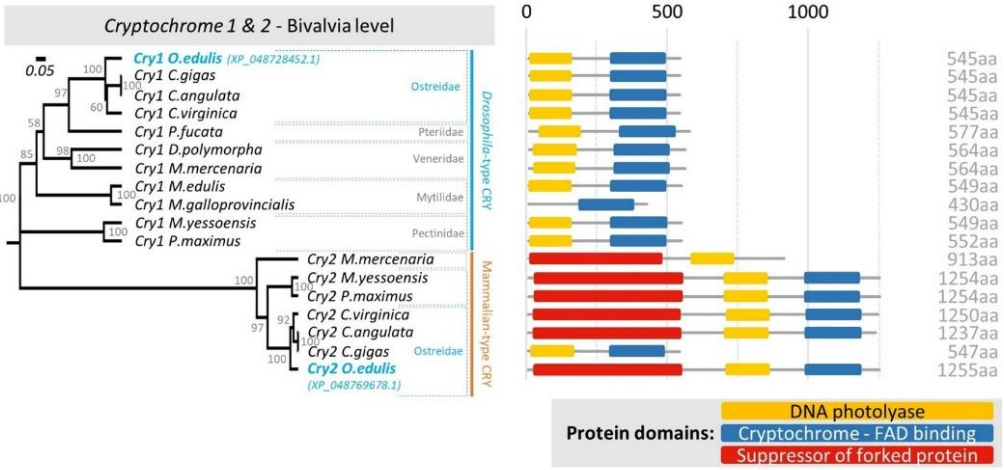
Timeless





Cryptochrome 1 & 2





HR3/ ROR

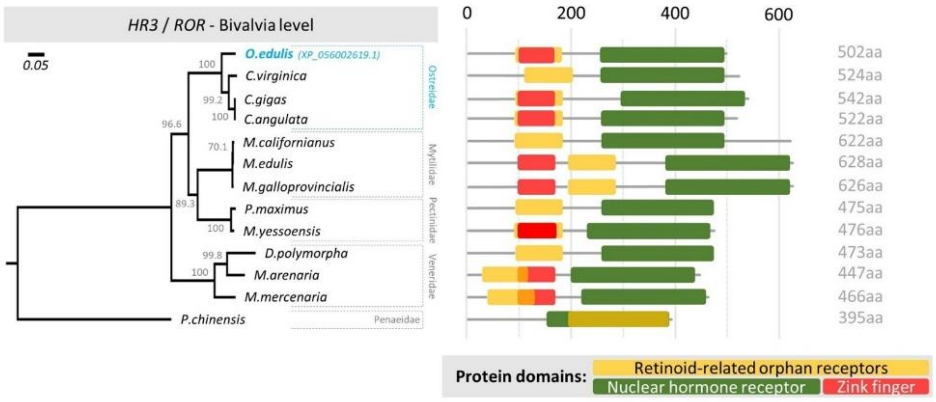


Figure S9.7. Phylogenetic position of *OeClock*, Oe*Bmal1, OePeriod, OeTimeless, OeCry1-OeCry2* and *OeRor* within the animal kingdom and the Bivalvia class and investigation of protein domains. Rooted phylogenetic trees of *Clock*, *Bmal1, Period, Timeless, Cryptochrome1-Cryptochrome2* and *ROR* sequences and protein domain comparison from various species within the animal kingdom and within the Bivalvia class. For the phylogenetic trees, the numbers on the branches represent bootstrap values provided by the Maximum Likelihood method with 1000 bootstrap replicates. Grey lines represent the protein length. Colored bars represent protein domains. Protein domains abreviations: basic helix-loop-helix (bHLH) domains, Per-Arnt-Sim (PAS) domains, Proline-Rich, Arabinogalactan Proteins, and Conserved Cysteines (PAC) domains, Timeless N-terminal (Timeless-N) domains, Timeless C-terminal (Timeless-C). Abbreviations for each species used in this figure: Acropora digitifera (A. digitifera), Acropora millepora (A. millepora), Apis mellifera (A. mellifera), Bombus affinis (B. affinis), Bombyx mori (B. mori), Bufo bufo (B. bufo), Crassostrea angulata (C. angulata), Crassostrea gigas (C. gigas), Crassostrea virginica (C. virginica), Danaus plexippus (D. plexippus), Danio rerio (D. rerio), Dreissena polymorpha (D. polymorpha), Drosophila melanogaster (D. melanogaster), Euphausia superba (E. superba), Eurydice pulchra (E. pulchra), Homo sapiens (H. sapiens), Mercenaria mercenaria (M. mercenaria), Mizuhopecten yessoensis (M. yessoensis), Mus musculus (M. musculus), Mytilus californianus (M. californianus), Mytilus edulis (M. edulis), Mytilus galloprovincialis (M. galloprovincialis), Mya arenaria (M. arenaria), Nematostella vectensis (N. vectensis), Octopus sinensis (O. sinensis), Ostrea edulis (O. edulis), Pecten maximus (P. maximus), Penaeus chinensis (P. chinensis), Penaeus japonicus (P. japonicus), Pinctada fucata (P. fucata), Platynereis dumerilii (P. dumerilii), Rana temporaria (R. temporaria), Rhincodon typus (R. typus), Sardina pilchardus (S. pilchardus), Tritonia tetraquetra (T. tetraquetra), Xenopus laevis (X. laevis).

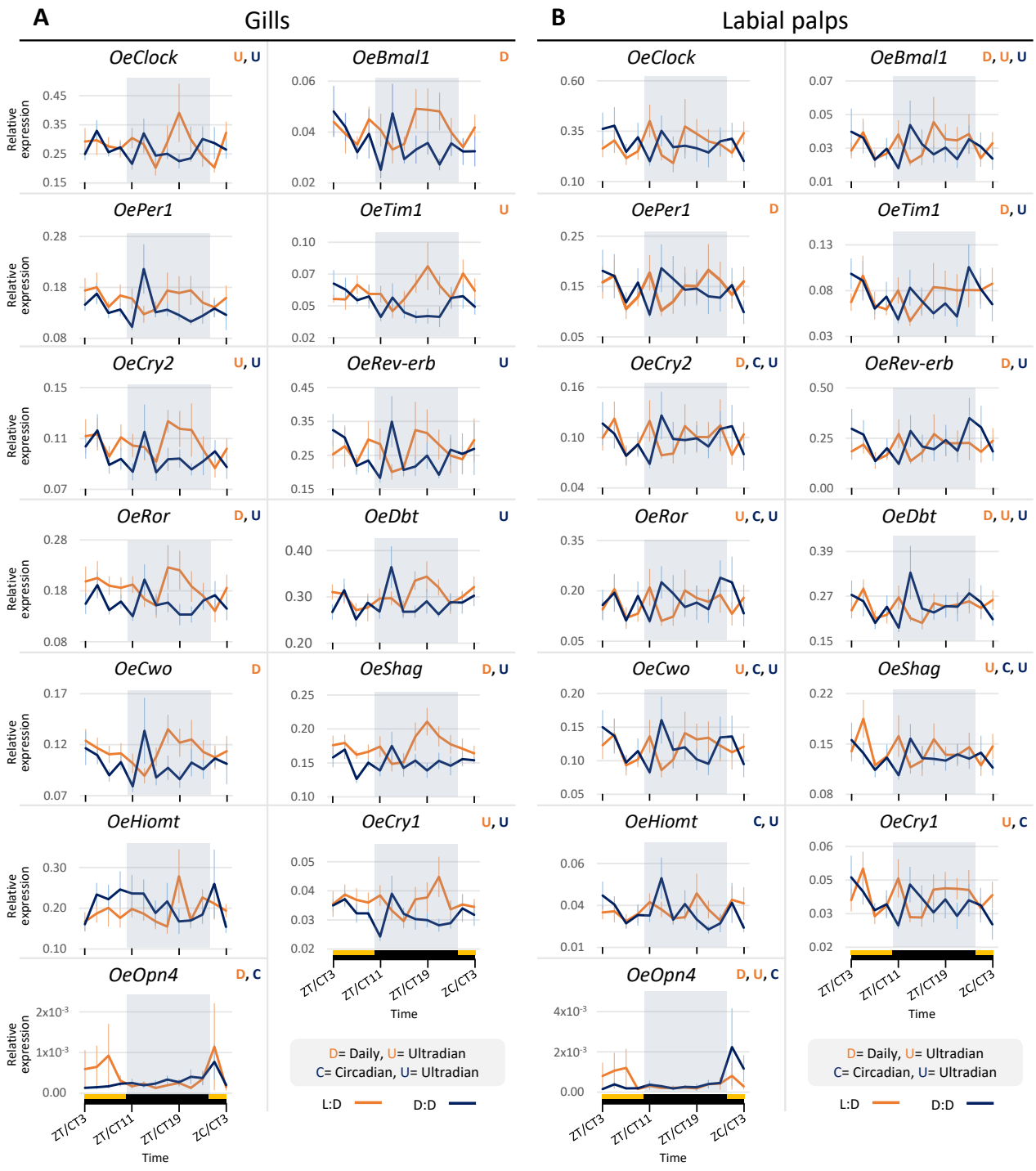


Figure S9.8. Temporal expression levels of genes involved in the clock machinery of *O. edulis* in gills and labial palps. Relative mRNA levels (mean ± SE, n = 8 oysters per time point and condition) of the circadian clock genes (*OeClock*, *OeBmal1*, *OePer1*, *OeTim1*, *OeCry2*, *OeRev-erb*, *OeRor*, *OeDbt*, *OeCwo*, *OeShag*), the gene related to

melatonin synthesis (*OeHiomt*), and the photoreceptor genes (*OeCry1* and *OeOpn4*) in gill tissues (A) and labial palps (B) during 24 hours of the L:D experiment (orange lines) and the D:D experiment (blue lines). Grey areas and bottom bars represent the scotophase of the L:D experiment (orange lines), while constant darkness was maintained during the D:D experiment (blue lines). ZT: *zeitgeber* time; CT: circadian time. Significant daily (D), circadian (C), or ultradian (U) expression patterns are indicated for each gene and experiment. Supplementary dataset S3 provides detailed gene expression data in gills and labial palps during L:D and D:D experiments. Supplementary table S2 provides detailed RAIN results.

9.3 Supplementary material for Article III

Table S9.3. Individual biometric data of oysters used in the behavioral experiment. Shell length and shell width measurements (mm) of the 16 *Ostrea edulis* individuals monitored continuously by HFNI valvometry throughout the study. Individual n°7 died a few days after deployment and was therefore excluded from the analyses.

Oyster n°	Length (mm)	Width (mm)
1	70	86
2	79	84
3	82	86
4	81	84
5	73	78
6	69	75
7	80	82
8	84	76
9	74	76
10	76	78
11	75	73
12	74	78
13	82	76
14	76	83
15	74	69
16	82	82

Table S9.4. Individual biometric data of oysters used in the molecular experiment. Shell length and shell width measurements (mm) of the 134 *Ostrea edulis* individuals collected during monthly sampling for gene expression analyses. Months indicate experimental sampling time points, with the exact sampling date provided in parentheses. Sampling dates correspond to standardized monthly sampling events scheduled according to the lunar phase.

Oyster n°	Length (mm)	Width (mm)
July 2023 (sampling date: 10 July 2023)		
1.1	75	75
1.2	70	75
1.3	70	68
1.4	75	65
1.5	75	80
1.6	70	65
1.7	90	80
1.8	75	70
August 2023 (sampling date: 11 August 2023)		
2.1	80	66
2.2	90	90
2.3	70	80
2.4	80	80
2.5	85	80
2.6	75	80
2.7	80	70
2.8	78	75
2.9	90	90
2.10	85	105
September 2023 (sampling date: 8 September 2023)		
3.1	95	95
3.2	105	90
3.3	85	80
3.4	95	90
3.5	80	78
3.6	90	90
3.7	85	85
3.8	100	80
3.9	90	98
3.10	95	85
October 2023 (sampling date: 6 October 2023)		
4.1	83	79
4.2	95	90
4.3	90	77
4.4	89	89
4.5	80	92

4.6	85	65
4.7	80	82
4.8	90	90
4.9	100	84
4.10	79	90
November 2023 (sampling date: 7 November 2023)		
5.1	85	85
5.2	85	85
5.3	95	90
5.4	90	80
5.5	85	75
5.6	95	80
5.7	85	80
5.8	85	60
5.9	90	90
5.10	90	90
5.11	95	95
December 2023 (sampling date: 6 December 2023)		
6.1	83	90
6.2	80	75
6.3	95	100
6.4	97	90
6.5	85	42
6.6	95	85
6.7	100	90
6.8	85	75
6.9	80	65
6.10	80	75
6.11	85	85
January 2024 (sampling date: 17 January 2024)		
7.1	80	67
7.2	85	75
7.3	85	75
7.4	100	80
7.5	77	75
7.6	85	80
7.7	80	75
7.8	80	80
7.9	75	67
7.10	75	75
February 2024 (sampling date: 12 February 2024)		
8.1	80	70
8.2	90	75
8.3	80	70
8.4	80	85

8.5	85	75
8.6	85	85
8.7	95	95
8.8	95	80
8.9	90	75
8.10	90	85
March 2024 (sampling date: 6 March 2024)		
9.1	75	70
9.2	80	65
9.3	70	80
9.4	85	75
9.5	90	75
9.6	95	75
9.7	80	70
9.8	70	65
9.9	85	65
9.10	85	75
April 2024 (sampling date: 3 April 2024)		
10.1	70	72
10.2	82	75
10.3	80	70
10.4	75	65
10.5	75	70
10.6	80	75
10.7	95	70
10.8	90	80
10.9	90	100
10.10	85	80
May 2024 (sampling date: 29 April 2024)		
11.1	85	85
11.2	100	95
11.3	95	80
11.4	85	85
11.5	80	85
11.6	80	75
11.7	85	75
11.8	80	75
11.9	80	85
11.10	90	85
11.11	80	85
June 2024 (sampling date: 30 May 2024)		
12.1	90	85
12.2	90	95
12.3	90	90
12.4	80	80

12.5	85	80
12.6	90	85
12.7	90	80
12.8	105	80
12.9	95	85
12.10	70	80
12.11	85	80
July 2024 (sampling date: 27 June 2024)		
13.1	85	80
13.2	80	80
13.3	85	70
13.4	80	80
13.5	80	80
13.6	95	95
13.7	90	80
13.8	90	70
13.9	80	75
13.10	85	80
13.11	100	90
13.12	100	80

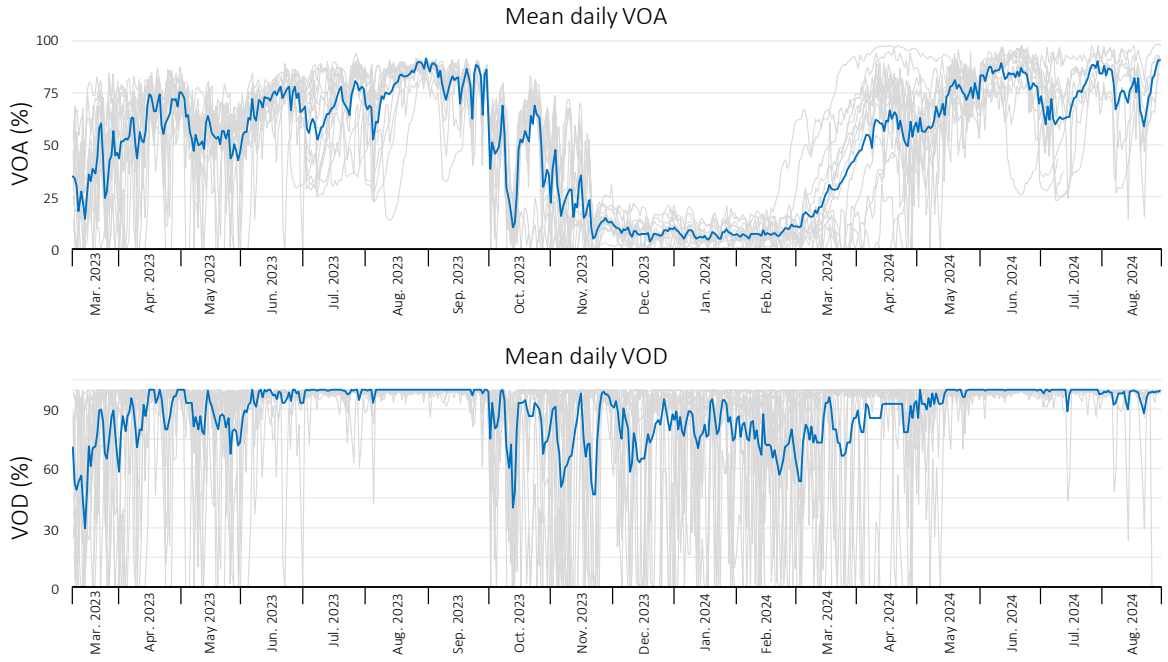


Figure S9.9. Mean daily valve opening amplitude (VOA, %) and valve opening duration (VOD, %) of *Ostrea edulis* at the individual level during our experiment on Helgoland. Grey lines represent the valve activity for each individual. The blue bold lines represent the daily mean.

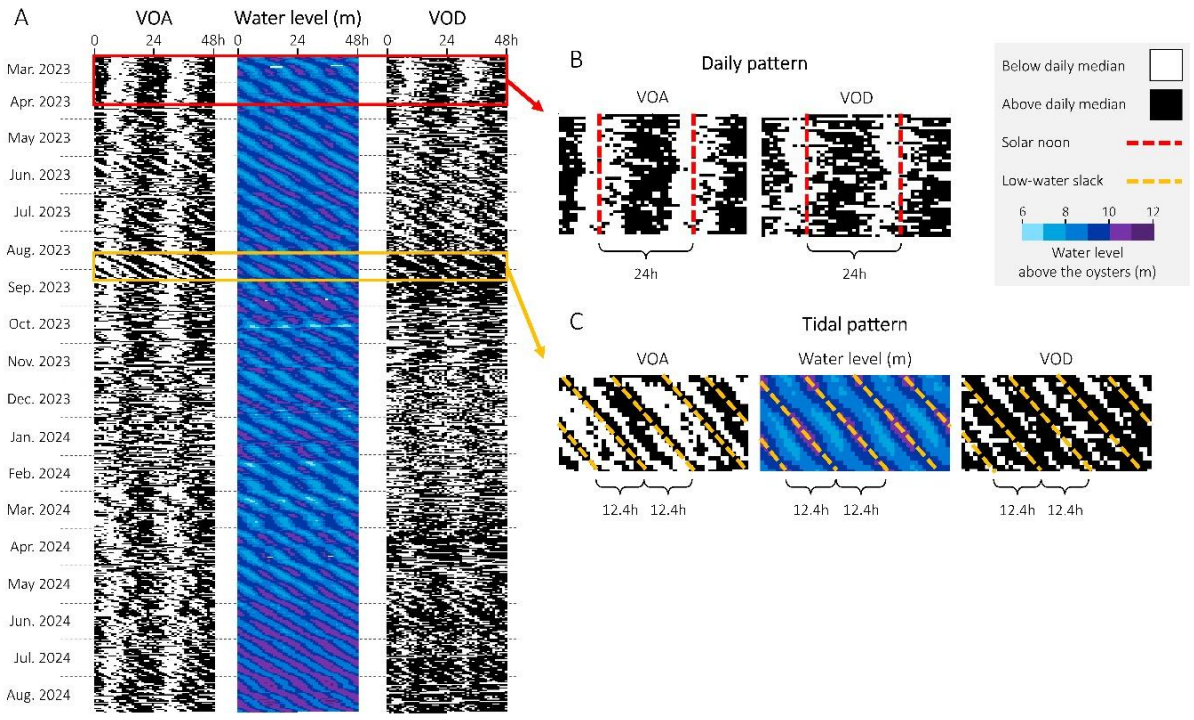


Figure S9.10. Visual representation of daily and tidal patterns in oyster valve activity. (A) Actograms of hourly mean valve opening amplitude (VOA, %) and valve opening duration (VOD, %) shown alongside the heatmap of the hourly water level above the oysters (m). (B) Representative example of the daily valve activity pattern showing the predominant 24h rhythmicity in VOA and VOD over several consecutive days. Red dashed lines indicate solar noon. (C) Representative example of the tidal valve activity pattern showing the predominant ~12.4h rhythmicity in VOA and VOD relative to water level over several consecutive days. Orange dashed lines indicate low-water slack.

Table S9.5. Accession number of identified genes of interest in *Ostrea edulis*. Abbreviations (Abbrev.) and accession number for housekeeping genes used for the molecular analysis are in blue.

Designation	Abbrev.	Size (aa)	Accession Number (NCBI)	Forward sequence for primer design	Reverse sequence for primer design
Core circadian clock genes					
OeClock	*OeClock*	677	XM_048900754.1	CAGCCACCAGTACCCAGAAT	TCTGGCTCGGAGATTTCTGT
OeBmal1	*OeBmal1*	546	XM_048874853.1	ACGAGAGGGTGCAGCTATGT	AGGATGTTCCGCACTGATTC
OePeriod	*OePer1*	1314	XM_048874757.1	GGCACATGCTGAACAAGAGA	AGAGGAAATGGTGGCACAAG
OeTimeless	*OeTim1*	949	XM_048891525.1	AGGAACCAGTGGCACGATAC	TGGGAAACACAAGACCAACA
Clock-related genes					
OeRev-erb	*OeRev-erb*	1089	XM_048918733.1	CTGTTCCGCCACAATTCTTT	CCTTGGACGCAATTGAGAAT
OeRor	*OeRor*	502	XM_048910521.1	CTCCCCAAGTCACCTCTCAG	GAGGGGTCATGCTGGATTTA
OeDoubletime	*OeDbt*	426	XM_048904376.1	GAATCGCTGGGATACGTGTT	CAGGGATCGGCAAAAGTTTA
OeShaggy	*OeShag*	412	XM_048923210.1	AGATCCAAACACCAGGTTGC	GATTGGTGTCTCCCGACAGT
OeClockWork Orange	*OeCwo*	376	XM_048885347.1	GCCCAAAGAACATTCAAGGA	GGTGCTTCTTCTGGAGCTTG
Clock-controlled metabolic gene					
OeNampt	*OeNampt*	468	XM_048882753.1	ACCCAAAATGCTTCTGGTTG	TGTTGAACCACGAAATCCAA
Gene involved in melatonin synthesis					
OeHiomt	*OeHiomt*	410	XM_048878998.1	TTGCAGAAGGATTGCAACAG	ATGATCATGGCATCGACAGA
Photoreceptor genes					
OeCryptochrome1	*OeCry1*	545	XM_048872495.1	GTCTCGCACACTCTGTGGAA	GTCCGTGAAGTCTGGGTCAT
OeMelanopsin	*OeOpn4*	384	XM_048908333.1	GACGAGGCTGGACAGAAAAC	GACGGTCCTGGTTGAGTGTT
Housekeeping genes					
OeGAPDH	*OeGapdh*	1317	XM_048908150.1	TGCAATCAATGATCCCTTCA	AGCCTTTCCGCTGATCACTA
OeEF1	*OeEf1*	1755	XM_048873495.1	CCAGGCCACAGAGATTTCAT	TTGACTCCGACAATGAGCTG
Oe28S	*Oe28S*	640	XM_048873299.1	TGGGGAAAGTGATAGACGTTG	AACCTCCTCCTTCCCAGTGT

9.4 Supplementary material for Article IV

Table S9.6. Quantitative details of female reproductive behavior in *Ostrea edulis*. Summary statistics are presented for each identified behavioral event (F1-F6), including male gamete uptake and ovary preparation (F1), spawning (F2), trans-ctenidial ovulation (F3), larval oxygenation (F4), onset of incubation (F5), and larval release/swarming (F6). "LS" indicates lost signal, and "No M1" refers to cases where no male spawning (M1) was detected at the time of F1.

	Incubation			Durations				Amplitudes			Spawning and Swarming characteristics				
Ind.	Incubation start (day)	F1 start (h)	Incubation end (day)	End of F6 (h)	F2 duration (min)	F6 duration (min)	Incubation duration (days)	Duration of the plateau during incubation (days)	Time between M1 and F1 (min)	VOA increase during F1 (%)	VOA difference between F2 and Incubation	VOA decrease during F3 (%)	VOA increase during F4 (%)	Contraction number during F2	Contraction frequency F2 (contraction/min)	Contraction number during F6	Contraction frequency F6 (contraction/min)
15	27/6/2023	21h15	13/7/2023	13h15	60	50	16.5	6	0	6	54	29	12	30	0.50	46	0.92
1	4/7/2023	17h05	16/7/2023	23h30	75	60	12	8	0	4	61	5	20	9	0.12	54	0.90
3	4/7/2023	17h05	17/7/2023	23h30	45	50	13	8	0	30	47	28	27	51	1.13	43	0.86
5	4/7/2023	17h05	17/7/2023	20h25	45	75	13	8	0	16	36	26	30	18	0.40	64	0.85
6	4/7/2023	17h05	17/7/2023	21h15	30	55	13	8	0	15	42	22	22	33	1.10	50	0.91
11	4/7/2023	17h05	17/7/2023	10h30	40	50	12.5	8	0	0	38	19	24	62	1.55	51	1.02
4	9/7/2023	19h20	21/7/2023	13h20	35	90	11.5	6	5	5	67	15	20	25	0.71	85	0.94
13	9/7/2023	19h30	21/7/2023	17h50	60	45	12	6	10	10	52	28	18	21	0.35	37	0.82
9	16/7/2023	15h30	29/7/2023	17h10	60	100	13	9	5	22	34	15	24	37	0.62	84	0.84
16	23/7/2023	21h40	5/8/2023	00h10	70	40	12	8	No M1	37	71	14	26	22	0.31	31	0.78
2	2/8/2023	22h55	14/8/2023	17h05	45	40	11.5	7	5	20	38	7	19	5	0.11	39	0.98
12	2/8/2023	23h	14/8/2023	14h40	45	45	11.5	7	0	10	58	21	17	43	0.96	51	1.13
10	12/8/2023	4h45	22/8/2023	14h30	35	45	10.5	7	No M1	15	50	30	35	20	0.57	38	0.84
3	9/9/2023	16h30	19/9/2023	19h50	40	60	10	4.5	0	20	48	24	16	42	1.05	54	0.90
15	16/6/2024	00h00	1/7/2024	17h20	60	80	15.5	11	10	15	78	55	35	45	0.75	67	0.84
4	1/7/2024	23h05	LS	LS	90	LS	LS	10	0	0	51	38	50	12	0.13	LS	LS
13	1/7/2024	23h05	15/7/2024	16h35	70	50	13.5	10	0	20	62	43	43	13	0.19	41	0.82
6	9/7/2024	4h30	21/7/2024	21h40	70	80	13	9	No M1	15	55	21	28	10	0.14	57	0.71
9	9/7/2024	5h10	LS	LS	70	LS	LS	LS	No M1	10	70	15	15	16	0.23	LS	LS
11	9/7/2024	4h45	LS	LS	150	LS	LS	9	No M1	15	52	12	14	57	0.38	LS	LS
1	17/7/2024	18h20	28/7/2024	20h05	60	110	11	6	0	5	60	20	32	5	0.08	77	0.7
14	8/8/2024	17h45	19/8/2024	4h55	45	40	10.5	6	No M1	15	60	20	38	14	0.31	38	0.95
13	18/8/2024	15h30	28/8/2024	16h45	45	60	10	7.5	0	5	50	32	20	21	0.47	51	0.85

Table S9.7. Quantitative details of male reproductive behavior in *Ostrea edulis*. Summary statistics are presented for the identified male spawning event (M1).

	Spawning		Duration	Spawning characteristics	
Ind.	M1 start (day)	M1 start (h)	M1 duration (min)	Contraction number during M1	Contraction frequency M1 (contraction/min)
14	27/6/2023	21h15	55	68	1.24
2	4/7/2023	17h05	25	47	1.88
8	4/7/2023	17h10	40	62	1.55
10	4/7/2023	17h10	55	85	1.54
14	4/7/2023	17h05	30	52	1.73
2	9/7/2023	19h10	50	40	0.80
8	9/7/2023	19h15	35	65	1.86
10	16/7/2023	15h25	55	130	2.36
4	2/8/2023	23h	15	50	3.33
8	16/8/2023	19h50	60	52	0.87
2	9/9/2023	16h30	60	25	0.42
2	15/6/2024	23h50	35	28	0.80
5	15/6/2024	23h50	30	44	1.47
8	15/6/2024	23h50	30	45	1.50
10	15/6/2024	23h50	25	45	1.80
16	15/6/2024	23h45	10	44	4.40
5	1/7/2024	23h05	20	26	1.30
8	1/7/2024	23h05	45	106	2.35
2	1/7/2024	23h05	15	27	1.80
10	17/7/2024	18h20	20	33	1.65
10	18/8/2024	15h30	10	14	1.40

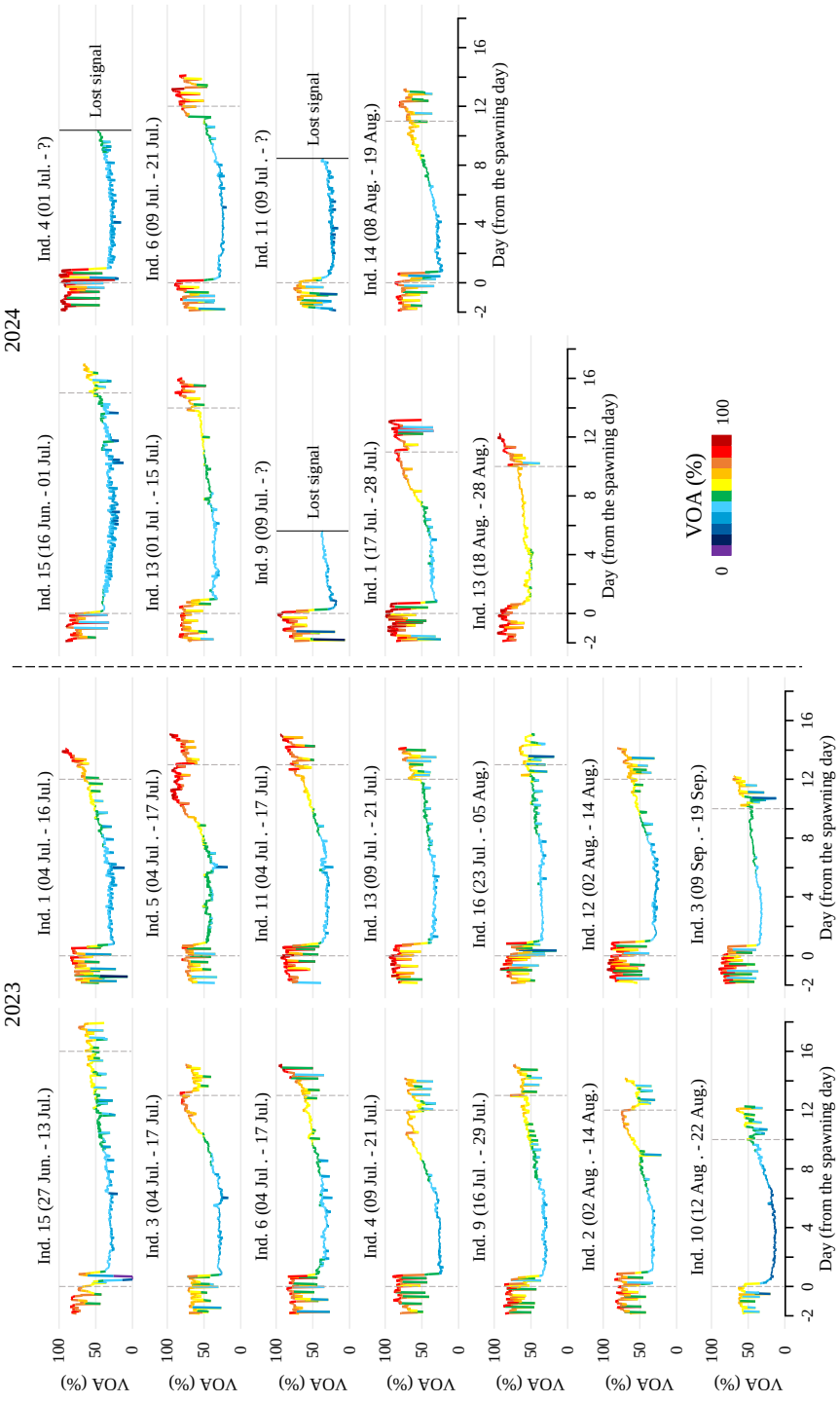


Figure S9.11. Valve opening amplitude (VOA, %) of *Ostrea edulis* during all identified female incubation periods in 2023 and 2024. Each line represents an individual, with color intensity reflecting VOA (%), highlighting clear reductions in valve activity corresponding to incubation events.

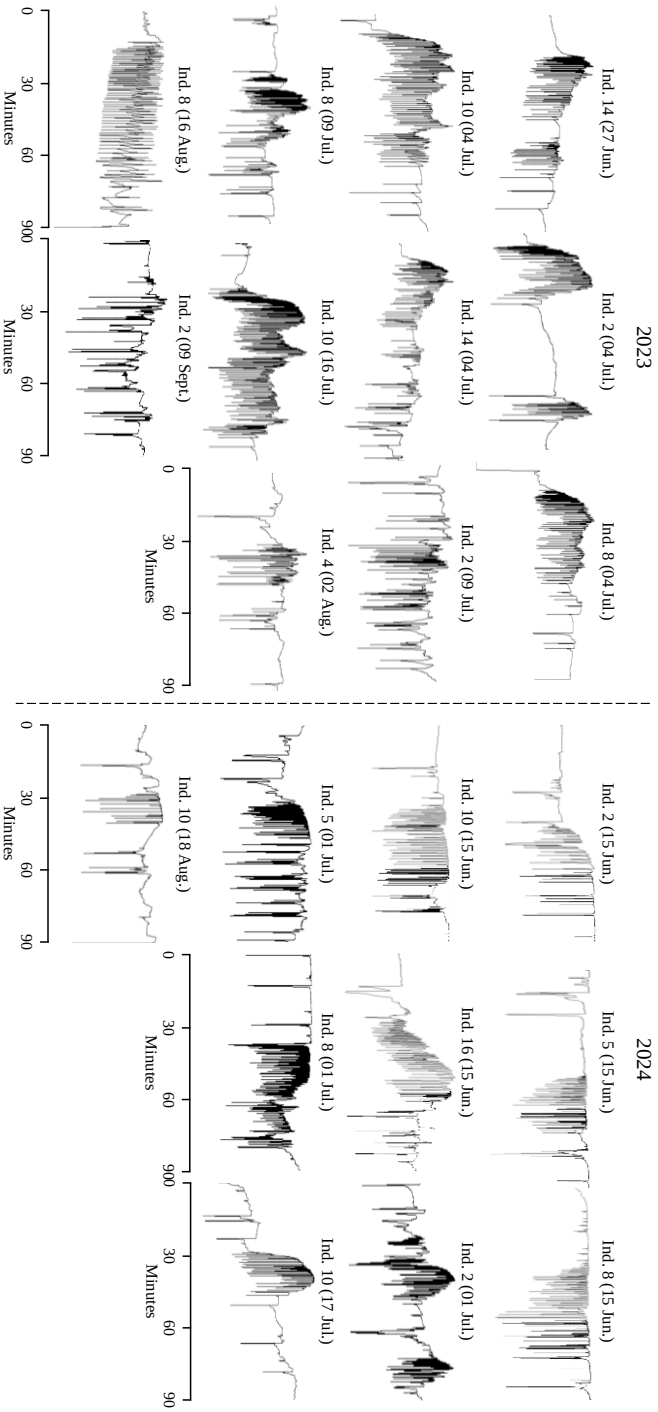


Figure S9.12. **Raw valve opening amplitude (VOA, %) of *Ostrea edulis* during all identified male spawning events (M1) in 2023 and 2024.** Each line represents an individual, showing the characteristic rapid adductor contractions associated with male gamete release.

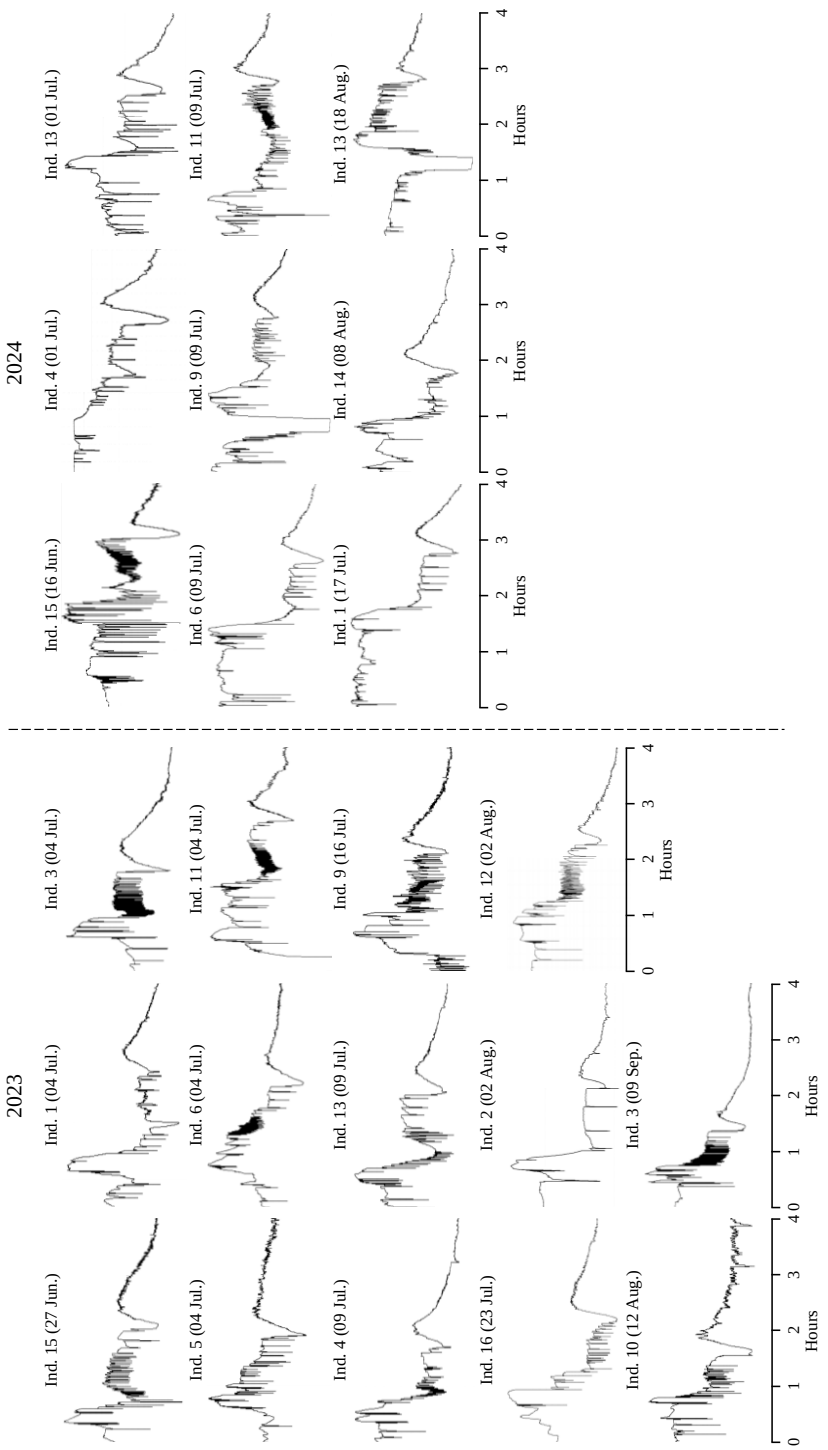


Figure S9.13. **Raw valve opening amplitude (VOA, %) of *Ostrea edulis* during all identified female ovulation events (F2) in 2023 and 2024.** Each line represents an individual, highlighting the rhythmic contractions associated with progressive egg transfer into the suprabranchial chamber.

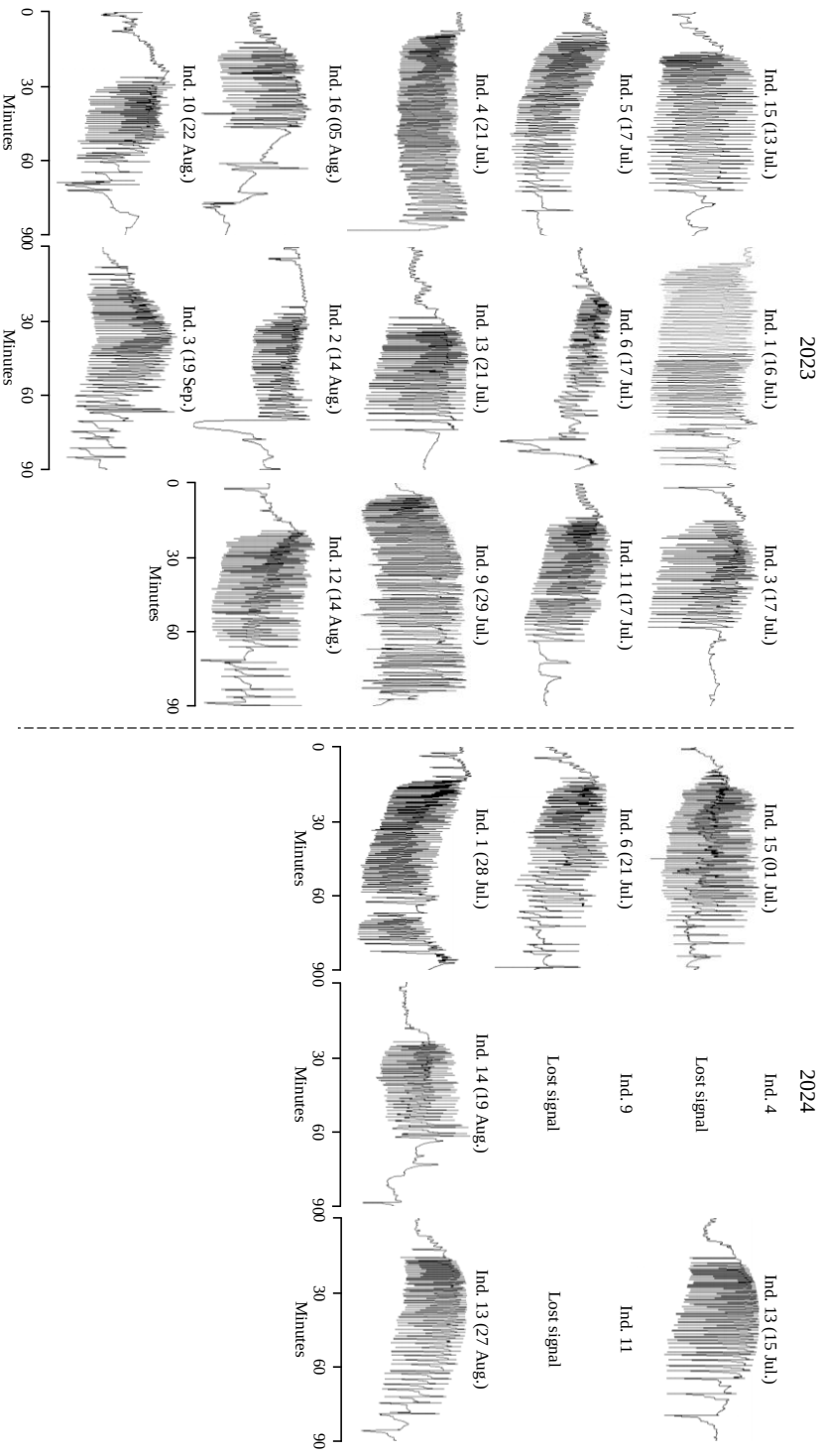


Figure S9.14. **Raw valve opening amplitude (VOA, %) of *Ostrea edulis* during all identified female swarming events (F6) in 2023 and 2024.** Each line represents an individual, illustrating strong and repetitive contractions leading to larval release.

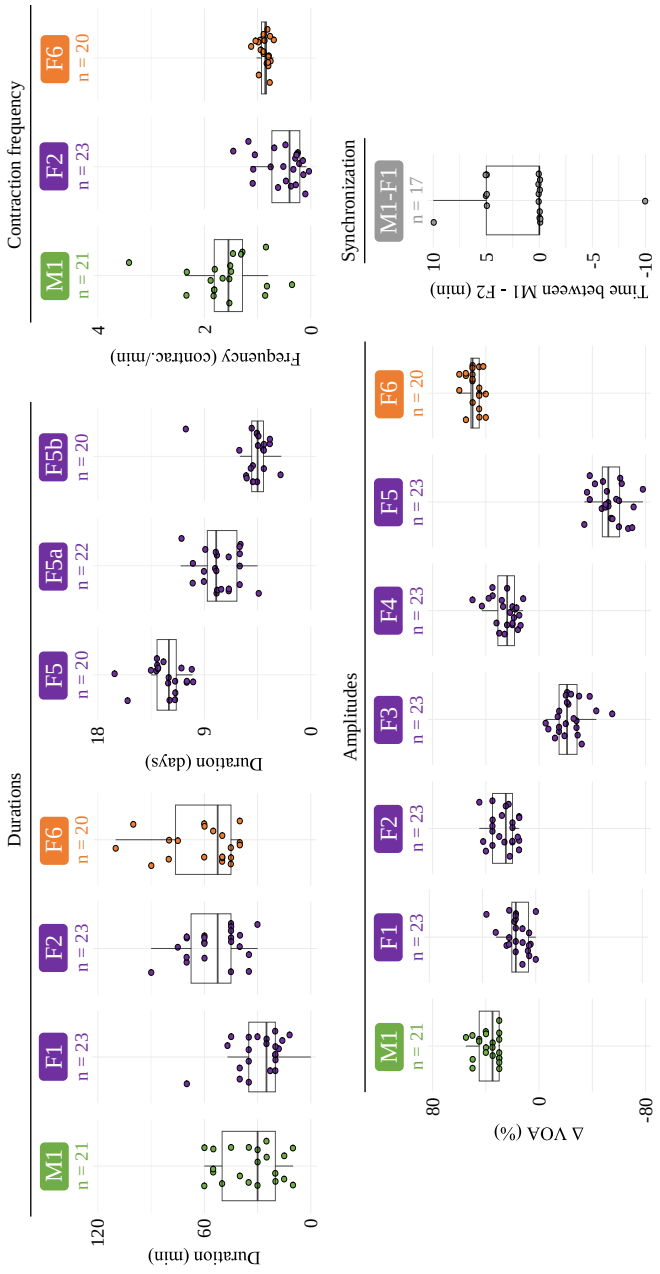


Figure S9.15. **Quantitative characteristics of reproduction events in *O. edulis*.** Boxplots summarize the distributions of event duration (M1, F1, F2, F5, F6; with F5 subdivided into F5a and F5b), contraction frequency (M1, F2, F6), ΔVOA (M1, F1-F6), and the temporal delay between M1 and F1. ΔVOA represents the difference between the minimum and maximum VOA recorded during each event. Data are derived from the measurements presented in Supplementary Tables S1-S2 and are shown as medians with interquartile ranges (Q1-Q3). A total of 44 spawning events were analyzed, including 25 in 2023 and 19 in 2024, comprising 23 female and 21 male spawning events.

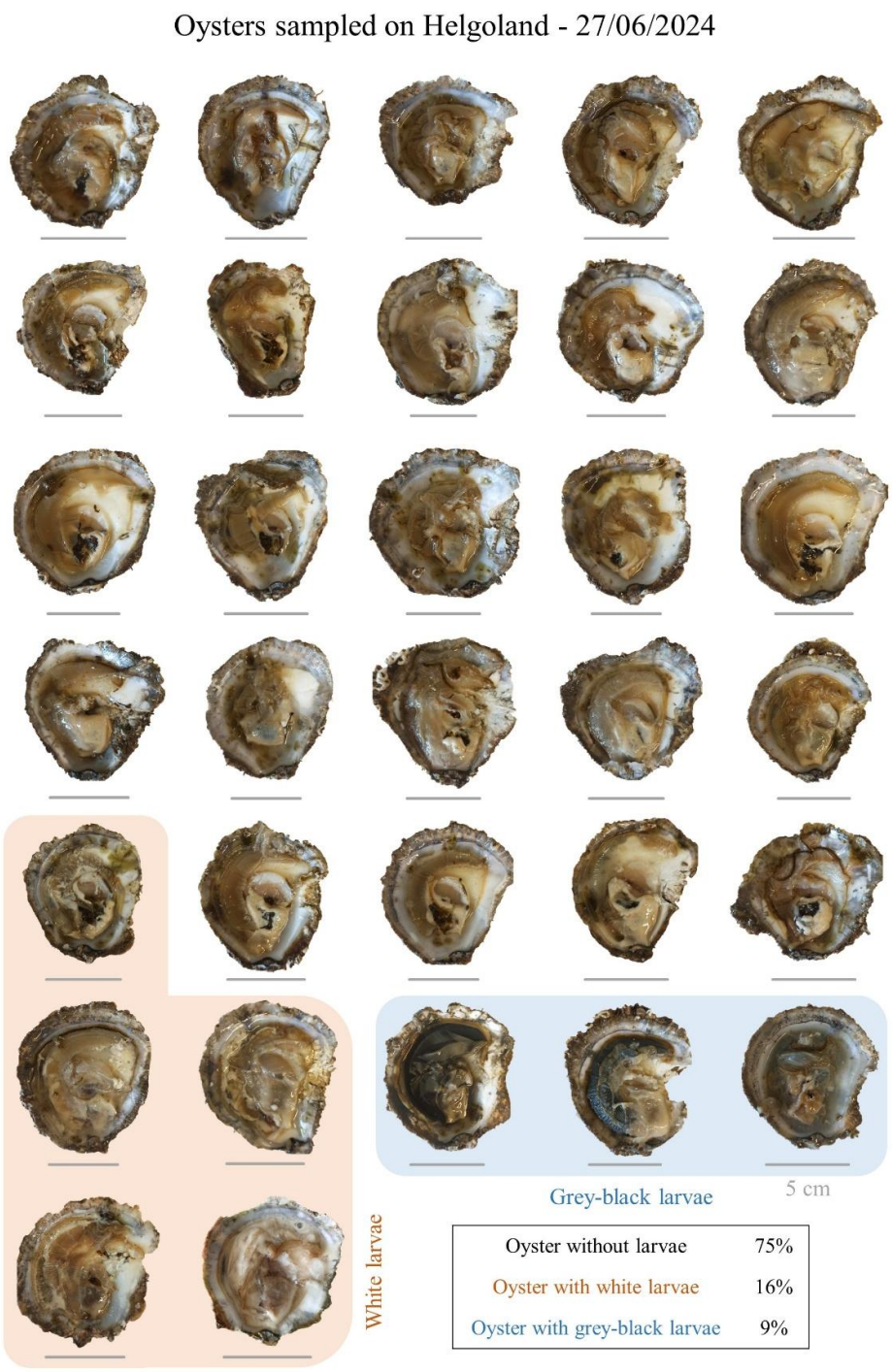


Figure S9.16. **Sampling of 33 *Ostrea edulis* on 27th June 2024 to identify incubation status.** Oysters containing early-stage larvae (white) are outlined in orange, while oysters with late-stage larvae (grey/black) are outlined in blue. Black lines indicate a 5 cm scale for each oyster. The figure also presents the statistics of oysters without larvae, with white larvae, and with grey/black larvae

Erklärung der Autorin

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ii. ii. die Dissertation bereits veröffentlichte bzw. zur Publikation eingereichte Teile enthält. Der Status der Publikationen ist zu Beginn der jeweiligen Kapitel angegeben.

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