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Review

# Molluscan bivalve settlement and metamorphosis: Neuroendocrine inducers and morphogenetic responses

Joyce A.*, Vogeler S.

*Department of Marine Science, University of Gothenburg, Sweden*

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## ABSTRACT

Surprisingly little is known about the nature and function of neurohormones in molluscs despite the fact that many of the neurotransmitters, including serotonin, catecholamines and estrogenic compounds that regulate reproduction in humans are also found in bivalve molluscs. Although mammalian and fish sex neurotransmitters have been comparatively well-studied, their role in molluscs is an emerging field where recent molecular and genetic research reveals an increasingly nuanced understanding of neuroendocrine pathways and morphogenetic processes. This knowledge is important in eco-toxicology given the prevalence of anthropogenic pollutants such as pharmaceuticals in wastewater, as well as pesticides and insecticides that are known to interfere with neuroendocrine signalling in a wide range invertebrate and vertebrate species. Knowledge of these pathways in molluscs is also important when developing anti-fouling compounds for marine applications. In the last few decades, the use of chemical inducers in bivalve aquaculture has transformed hatchery technology not only by increasing survival rates at metamorphosis, but also by using neurohormones to create 'cultchless' or 'single-seed' spat in some species (e.g. oysters) to bypass normal pathways that involve attachment to a substrate. Hatchery protocols for inducing settlement and metamorphosis with a range of chemical inducers in commercial shellfish species have been developed primarily from empirical studies based on trial and error, but such an approach is not reliably effective in all species, and trial and error is particularly time-consuming when developing protocols for new species. During the transition from larval to juvenile to adult life stages, neuroendocrine and immune functions mediate complex metabolic processes, and recent research on hematopoiesis, immune-competence, neuroendocrine pathways and gene regulation give insights into these complexities. In this review, we provide a current overview of research regarding the neuroendocrine basis for physiological mechanisms involved in bivalve settlement and metamorphosis, given it is a period when hatcheries often experience the greatest losses. Despite a plethora of empirical studies on specific species, the processes and neurotransmitters involved are surprisingly not well explored. We focus on metamorphosis as a key developmental period by highlighting some of the most promising new research in this area, with a focus on the value of these insights for commercial applications in aquaculture.

## 1. Introduction

Most of our current understanding of neuroendocrine pathways, transmembrane receptors and their roles in metamorphosis of molluscan species is based on models of vertebrates and insects, areas that have been comparatively well studied. Metamorphosis, the transition of free-swimming larvae to benthic and often sessile and attached juveniles, is one of the most distinctive life changing events in many molluscan species. Much of the research on metamorphosis in invertebrate species has emerged from the ecotoxicology literature and is focused on introduced sources of neurohormones in the environment from contaminants that have the potential to disrupt reproductive physiology and development in a wide range of species (Lyssimachou et al., 2015; Waye and Trudeau, 2011). The effectiveness of many insecticides, for instance, relies on their ability to target metamorphosis and moulting in insects (Palli and Cusson, 2007); similarly, many pharmaceuticals (e.g. birth control pills, drugs for degenerative and psychiatric disorders) are designed to act as neurotransmitters or hormone substitutes (Stromgaard et al., 2009). Given that endocrine pathways are highly conserved among unrelated species, there is a worldwide concern about residues of such compounds in the environment because of their persistence and effects on survival of non-target species (Bergman et al., 2013). For instance, increases in imposex and skewed sex ratios in molluscs have been linked to endocrine disrupting pollutants from

* Corresponding author.
*E-mail address:* alyssa.joyce@gu.se (A. Joyce).

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**Glossary**

| | |
|---|---|
| 20E | 20-hydroxyecdysone |
| 5-HT | serotonin or 5-hydroxytryptamin |
| ACh | acetylcholine |
| AChR | acetylcholine receptor |
| ACTH | adrenocorticotropichormone |
| ASO | apical sensory organ |
| ATP | adenosine triphosphate |
| cAMP | cyclic adenosine monophosphate |
| DA | dopamine |
| DβH | dopamine-β-hydroxylase |
| EcR | ecdysone receptor |
| EPI | Epinephrine |
| GABA | gamma-amino-butyric acid |
| GPCR | G-protein coupled receptors |
| IBMX | iso-butyl-methyl-xanthine |
| L-DOPA | L-3,4-dihydroxypheylalanine |
| LGC | light-green cells |
| LPS | lipopolysaccharide |
| MIP | molluscan insulin-related peptide |
| MSH | melanocyte-stimulating hormones |
| NE | norepinephrine |
| NEI | neuroendocrine-immune |
| OA | octopamine |
| pf | post fertilisation |
| PNMT | phenylethanolamine-*N*-methyltransferase |
| PO | phenoloxidase |
| POMC | pro-opiomelanocortin |
| pro-PO | pro-phenoloxidase |
| pro-PTTH | pro-prothoracicotropic hormone |
| SMPs | shell matrix proteins |
| TEA | tetraethylammonium |
| TH | tyrosine hydroxylase |
| TNF | tumor necrosis factor |
| TPH | tryptophan hydroxylase |
| TY | tyrosine |

anthropogenic sources (Horiguchi, 2006; Ketata et al., 2008; Matthiessen and Gibbs, 1998; Oehlmann et al., 2007). As molluscs are key species in marine ecosystems, breeding failures due to these pollutants are of concern due to their cascading effects on marine ecosystems.

A better understanding of bivalve neuroendocrinology is not only important in relation to anthropogenic pollutants, but is also of commercial interest because of the very practical applications of this knowledge when developing anti-fouling agents. In the same way that many insecticides are designed to disrupt important developmental pathways in arthropods, many anti-fouling agents used in the marine environment have significant commercial value because of their ability to disrupt metamorphosis, and thus the undesirable attachment of invertebrates to ships and structures (Chen and Qian, 2017; Qian et al., 2015). The cost of fouling to the shipping industry is significant, in the order of a hundred billion dollars a year, thus the efficiency of antifouling compounds can always be improved. Antifouling, especially to reduce 'hard' (calcareous) fouling involving barnacles and bivalves is particularly important given that this is often the most problematic to remove, and costliest to the shipping industry because of induced drag. Attachment of molluscs to structures is not only a problem for shipping, but also represents a significant challenge for a myriad of other industries, including oil rigs and other underwater construction, as well as industries that require high volume water intakes in their production processes (e.g. pulp and paper manufacturing, many food processing and desalination plants).

One of the most important applications of current knowledge about metamorphic pathways in molluscs, and the focus of this review paper, is within the aquaculture industry, where metamorphosis is a period of significant loss in hatcheries. Efficiency in being able to induce synchronous metamorphosis, as well as the ability to ensure high survival rates during metamorphosis, are both very important to bivalve (e.g. clam, mussel, scallop, oysters) and gastropod (e.g. abalone, locos) hatcheries. The need for aquaculture products with uniform shape and size for ease of handling has increasingly pushed the development of specific hatchery and grow-out technologies that rely on consistent and predictable metamorphosis, and the creation of 'single-seed'. In the past, for species that attach through cementing to a substrate (e.g. oysters), a suitable substrate was added to a hatchery tank when larvae were deemed ready to metamorphose (e.g. ground shell or sand known as 'cultch', rope for mussels, or various types of 'settlement' plates). Given that non-attached spat is a desirable outcome for some types of grow-out systems, the ability to manipulate neuroendocrine pathways to produce 'cultchless' single seed has become popular even though the pathways and mechanisms involved are not understood. More reliable and effective ways of predicting competence ('readiness to set') and/or preventing attachment to substrates are definitely possible based on insights into neuroendocrine-immune (NEI) gene activity that accompanies larval development. To our knowledge, there has been no review to-date that provides a synthesis of what is currently known about metamorphic processes in bivalves (and gastropods, in cases where little is known about bivalves). Thus in the following sections we focus on this key developmental stage to highlight some of the most promising new research with a focus on the value of these insights for commercial aquaculture.

## 2. Context

In some species, phenotypic characteristics indicating readiness to set may be clear indicators for hatchery personnel to 'set' a batch of larvae. For instance, in certain bivalves such as oysters or mussels where there is a large, visible eyespot, the appearance of the eyespot in the pediveliger stage in conjunction with foot extension (seeking behaviour), typically indicates a point at which exposure to exogenous stimuli (e.g. cholinergic or catecholaminergic compounds, light exposure, ionic changes) may induce greater numbers of metamorphosed larvae. Hatchery personnel can generally recognize these stages and rely on these types of phenotypic indicators to guide them when treating batches with chemical stimulants in order to achieve synchronous metamorphosis. Problems arise when working with new species for which there are indeterminate physiological indicators for readiness to set or lack of prior empirical studies on chemical induction. Energy expenditures at this life stage are significant, hence misjudging these cues and inducing too early leads to mortalities; waiting too long results in attachment to the sides of tanks, or failure to develop in non-attached species with resultant lowered survival. Since this stage is sensitive to poor environmental conditions, presence of pathogens and insufficient energy reserves can all dramatically affect survival rates. Although higher degrees of mortality are to be expected during early life stages in most broadcast spawners, metamorphosis represents one of the most significant periods of mortality for many hatcheries, thus making it imperative to better understand the underlying processes and develop better strategies for managing this developmental stage.

Hatchery terminology for metamorphic competence can be confusing. Metamorphosis in many bivalve and gastropod species is an irreversible physiological process that includes a change from a free living larva to a predominantly sessile juvenile, including loss of the velum, development of gills, and production of adult shell. After