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ORIGINAL ARTICLE

Larval settlement and metamorphosis of the invasive biofouler, *Mytilopsis sallei*, in response to ions and neuroactive compounds

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ABSTRACT

In this study, we investigated larval settlement and metamorphosis of the invasive fouler *Mytilopsis sallei* exposed to ions, neurotransmitters and blockers inhibiting their respective actions. Excess K^+ effectively induced larval settlement and metamorphosis, while the voltage-gated potassium channel blocker, TEA, significantly inhibited the K^+ inducing effect, suggesting that a voltage-gated potassium channel may play a role in *M. sallei* settlement and metamorphosis. Excess Ca^{2+} did not induce larval settlement and metamorphosis, while Mg^{2+} and NH_4^+ inhibited both. Among the neurotransmitters, GABA did not induce *M. sallei* larvae to settle and metamorphose at 10^{-6}–10^{-4} M concentrations, while 5×10^{-5}–10^{-4} M L-DOPA (a dopamine precursor), 5×10^{-6}–10^{-4} M dopamine (an epinephrine precursor) and 5×10^{-5}–10^{-4} M epinephrine significantly induced larval settlement and metamorphosis, indicating the presence of an epinephrine biosynthesis pathway in this species and its role in the regulation of larval settlement and metamorphosis. Furthermore, the inducing effect of dopamine on *M. sallei* settlement and metamorphosis was inhibited by SCH23390, a selective D1 dopamine receptor antagonist. Similarly, the inducing effect of epinephrine was inhibited by chlorpromazine, an α_1-adrenergic antagonist, suggesting that the D1 dopamine receptor and α_1-adrenoceptor may play active roles in the processes of settlement and metamorphosis of *M. sallei* larvae. Here, we have shown for the first time the responses of larval settlement and metamorphosis of dreissenid mussels to pharmacologically active compounds. The results provide new insights into the biochemical mechanisms underlying larval settlement and metamorphosis of *M. sallei*, which may be useful to develop effective strategies to control this invasive fouling organism.

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Introduction

Many marine invertebrate species have larvae that remain planktonic for various durations ranging from minutes to months. Then, they settle and metamorphose into benthic juveniles (Hadfield & Paul 2001; Clare 2011). Larval settlement and metamorphosis are not only critical for juvenile growth, but also for adult reproduction (Pawlik 1992), affecting population and community dynamics (Connell 1985). A number of studies have shown that natural and artificial chemical cues influence the larval settlement and metamorphosis of marine invertebrates (Crisp 1984; Pawlik 1992; Hadfield & Paul 2001; Prendergast 2010). However, only a few natural chemical inducers that influence larval settlement and metamorphosis have been fully isolated and chemically characterized (Yvin et al. 1985; Pawlik 1986; Tsukamoto et al. 1999; Swanson et al. 2004; Dreanno et al. 2006; Tebben et al. 2011). Thus, further understanding of the mechanisms that regulate larval settlement and metamorphosis has been limited. Alternatives to natural cues are pharmacological compounds such as agonists and antagonists capable of activating or blocking specific signal transduction pathways. Such studies could also help understanding of the molecular mechanisms associated with larval settlement and metamorphosis (Kimura et al. 2003; Yang et al. 2008, 2011, 2013; Young et al. 2011, 2015; Gohad et al. 2012; Wang et al. 2015). Furthermore, the information is also important for identifying potential inducers and inhibitors of larval settlement and metamorphosis for application in aquaculture (Young et al. 2011; Yang et al. 2013; Wang et al. 2015) and antifouling (Qian et al. 2013; Almeida & Vasconcelos 2015).

A number of compounds have been found to affect the settlement and metamorphosis of marine invertebrates. These include catecholamine, choline

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derivatives, amino acids and derivatives, and inorganic ions in bivalves (Yang et al. 2013, 2014; Wang et al. 2015; Young et al. 2015), barnacles (Gohad et al. 2012; Jin et al. 2014), bryozoans (Yu et al. 2007), polychaetes (Jin & Qian 2004) and ascidians (Kimura et al. 2003; Zega et al. 2005). However, the responses of larvae to pharmacological compounds are highly variable among species. For instance, γ-aminobutyric acid (GABA) was shown to induce larval metamorphosis in the mollusc *Mytilus unguiculatus* Valenciennes, 1885 (Yang et al. 2013), but had no significant effect on the larval metamorphosis of *Ostrea angasi* G.B. Sowerby II, 1871, another mollusc (O'Connor et al. 2009).

Invasive dreissenid mussels are considered serious pests because they have caused adverse ecological impacts and economic problems by invading new environments and fouling submerged structures (Higgins & Zanden 2010; Kennedy 2011; Matthews et al. 2014). It has been suggested that dreissenids are transported on ship hulls where they attach or as planktonic larvae in ballast water. When they are thus introduced to new regions, they establish as fouling organisms (Morton 1981; Johnson & Carlton 1996; Chu et al. 1997). Therefore, it is critical to understand the population recruitment of invasive dreissenids to better control these nuisance mussels. However, thus far, the effects of pharmacological compounds on the larval settlement and metamorphosis of invasive dressenids have not been investigated.

The Caribbean false mussel *Mytilopsis sallei* (Récluz, 1849) is an invasive dreissenid species that was introduced into the Pacific via the Panama Canal (Morton 1981). High-density populations of this species have been found in Southeast Asia (Tan & Morton 2006; Liao et al. 2010; Wong et al. 2011), including the southeastern coast of mainland China (Cai et al. 2006). This dreissenid species has been reported to impact the community structure of fouling macrofauna and reduce species diversity during the summer periods in Yundang Lagoon, Xiamen, China (Cai et al. 2014). In this study, we examined the responses of larval settlement and metamorphosis of *M. sallei* to inorganic ions, neurotransmitters and blockers inhibiting their respective actions.

Material and methods

Larval culture

Adults of *Mytilopsis sallei* were collected from the submerged ropes of a fish farm in Maluan Bay, Xiamen, China (24°33′N, 118°01′E). Spawning induction and larval culture were carried out in the laboratory as described by He et al. (2015). Briefly, mussels were exposed to air overnight and then placed in warm filtered seawater (UV-treated, temperature 32°C, 20 psu). The seawater was aerated vigorously during spawning induction. Fertilized eggs were washed through a 100-μm mesh net and subsequently a 30 μm mesh net to remove extraneous tissue and excess sperm. Embryos and larvae were incubated at a density of 3–5 individuals ml^{-1} in filtered seawater (FSW, 20 psu) at 27 ± 1°C. The water was aerated gently and changed daily. After incubation for 24 h, veliger larvae were obtained and fed with *Dicrateria zhanjiangensis* Hu (Chrysophyta) at 0.5–1 × 10^5 cells ml^{-1} day^{-1}. After 6–8 days of incubation, pediveliger larvae (Figure 1a) with a shell length of 232.8 ± 37.1 μm were harvested using a 200 μm mesh net and used for the bioassays. Pediveliger larvae of *M. sallei* were able to swim in the seawater and crawl and explore on the substrate for short intervals with competence to settle and metamorphose (He et al. 2015).

Treatment solutions

The alkali ions tested in the study included potassium, calcium, magnesium and ammonium. The neurotransmitters tested were GABA, L-3,4-dihydroxyphenylalanine (L-DOPA), dopamine and epinephrine, which have been reported to affect larval settlement and metamorphosis in many species of marine invertebrates (Zega et al. 2005; Yu et al. 2007; Yang et al. 2013, 2014; Wang et al. 2015). The blockers used in the study included tetraethylammonium (TEA) that blocks the potassium channel, L-3,4-dihydroxyphenylalanine *R*(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (SCH23390) and sulpiride that block dopamine receptors, and chlorpromazine, yohimbine, atenolol and butoxamine that block epinephrine receptors. The names, manufacturers, stock concentrations and test concentrations of the compounds are shown in Table I. Stock solutions of alkali ions, TEA, dopamine hydrochloride, GABA, yohimbine hydrochloride and chlorpromazine hydrochloride were prepared by directly dissolving them in FSW (20 psu), while stock solutions of L-DOPA, epinephrine, SCH23390, sulpiride, atenolol and butoxamine were prepared by dissolving them in FSW with 0.1 ml of 1 M HCl. For each compound, stock solution and test solutions were freshly prepared prior to the experiments. Test solutions were prepared by dilution

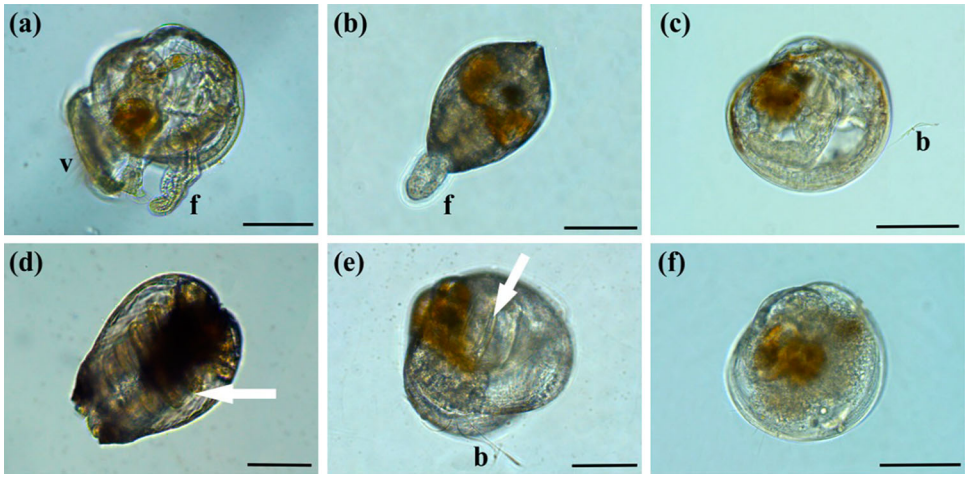

Figure 1. *Mytilopsis sallei* pediveligers that were swimming (a, competent to settle and metamorphose), settled (b, vertical view; c, lateral view), metamorphosed (metamorphosed into plantigrade: d, vertical view; e, lateral view) and dead (f). Abbreviations: b, byssus; f, foot; v, velum. Arrows indicate gill filaments. Scale bar: 100 μm.

of stock solutions with FSW to achieve the desired concentrations (Table I).

Bioassays of larval settlement and metamorphosis

The bioassays were conducted in sterile six-well polystyrene petri plates. Three replicates were set up for each treatment. In each replicate, 30–40 pediveliger larvae of *Mytilopsis sallei* were added per well containing 10 ml of a test solution. A previous pilot study had shown that larval density in the range of 1–6 larvae ml^{-1} had no significant effect on larval settlement, metamorphosis and mortality in *M. sallei*. Petri plates were maintained at 27°C in the dark during the experiments. A schematic presentation of the experimental design is shown in Figure 2.

To bioassay alkali ions, pediveligers were continuously exposed to the test solutions of alkali ions throughout the experimental period. Controls (10 ml FSW) were used in all bioassays. To understand the effects of KCl on settlement and metamorphosis of *M. sallei* larvae, the larval response to TEA, a potassium channel inhibitor, was investigated. The pediveliger larvae were placed in FSW containing 9 mM KCl and TEA at different concentrations. In the control, larvae were exposed to FSW containing only 9 mM KCl. In the experiments with alkali ions and TEA, larval settlement, metamorphosis and mortality were observed after 24, 48 and 72 h of exposure through a Leica microscope (DM IL LED) to test solutions.

In the bioassays of neurotransmitters (including GABA, L-DOPA, dopamine and epinephrine), pediveligers were exposed to the test solutions of neuroactive compounds for 2 h, then rinsed three times with FSW and finally transferred to wells containing 10 ml FSW each. After 12, 24 and 48 h of incubation, larval settlement, metamorphosis and mortality were observed. Control larvae exposed to 10 ml FSW throughout the experiment were also observed at the same time points.

To explore the involvement of dopamine receptors and adenoreceptors in *M. sallei* larval settlement and metamorphosis, larval responses to two dopamine receptor antagonists (SCH23390 and sulpiride) and four adrenergic antagonists (yohimbine, chlorpromazine, atenolol and butoxamine) were also investigated. In each treatment, larvae were first incubated in 9 ml antagonist solutions for 15 min. Then, 1 ml dopamine stock solution (10^{-3} M) was added to each solution with dopamine receptor antagonists, and 1 ml epinephrine stock solution (10^{-3} M) was added to each of the adrenergic antagonists solutions so that the final concentration of both compounds was 10^{-4} M. After a 2 h exposure, larvae were rinsed three times in FSW and transferred to wells containing 10 ml FSW. Control larvae were exposed to 10^{-4} M dopamine or epinephrine for 2 h and then transferred to FSW. After 12, 24 and 48 h of incubation, larval settlement, metamorphosis and mortality were observed.

According to the descriptions in the relevant literature (Bonar et al. 1990; Ke et al. 1994; Beiras & Widdows 1995; García-Lavandeira et al. 2005; Grant et al. 2013), larval settlement was confirmed by crawling with an extended foot or attaching by byssus while the velum is absorbed (Figure 1b,c); larval metamorphosis was confirmed by loss of the velum, the appearance of mature gill filaments and the appearance of a shell morphologically similar to that of adult mussels (Figure 1d,

Table I. Stock and test concentrations of the chemical compounds assayed.

Chemical compound	Manufacturer	Stock concentration (M)	Test concentration (M)		
KCl	SCRC (Shanghai, China)	0.15	0.003 0.012	0.006 0.015	0.009
$CaCl_2$	SCRC (Shanghai, China)	0.25	0.01 0.025	0.015	0.02
$MgCl_2$	SCRC (Shanghai, China)	0.5	0.001 0.05	0.01	0.03
NH_4Cl	SCRC (Shanghai, China)	0.5	5×10^{-4} 5×10^{-2}	1×10^{-3}	1×10^{-2}
TEA	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-5}	5×10^{-5}	1×10^{-4}
Epinephrine	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
Dopamine hydrochloride	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
L-DOPA	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
GABA	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
SCH23390	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
Sulpiride	TCI (Toshima, Japan)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
Yohimbine hydrochloride	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
Chlorpromazine hydrochloride	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
Atenolol	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}
Butoxamine	Sigma (St Louis, MO, USA)	10^{-3}	1×10^{-6} 5×10^{-5}	5×10^{-6} 1×10^{-4}	1×10^{-5}

TEA, tetraethylammonium; L-DOPA, L-3,4-dihydroxyphenylalanine; GABA, γ-aminobutyric acid; SCH23390, *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; SCRC, Sinopharm Chemical Reagent Co. (Shanghai, China); Sigma, Sigma Chemical Co. (St Louis, MO); TCI, Tokyo Chemical Industry Co. (Toshima, Japan).

e); larvae that showed no signs of movement of the velum, foot, or gut while the tissue inside the shell became ulcerated were considered dead (Figure 1f).

Statistical analysis

Results were analysed with SPSS 17.0 software. Percentages of larval settlement, metamorphosis and mortality were arcsine-transformed prior to analysis by a one-way analysis of variance (ANOVA) with Tukey's HSD. The significance level was set at $P < 0.05$.

Results

Larval settlement and metamorphosis in response to alkali ions

The effects of alkali ions on larval settlement and metamorphosis of *Mytilopsis sallei* are shown in Figure 3. Larval settlement was significantly induced by 6 and 9 mM K^+ after a 72 h exposure. The treatments at 6–12 mM K^+ concentrations also exhibited significantly higher percentages of larval metamorphosis than the control after 24, 48 and 72 h. However, when the K^+ concentration was as high as 15 mM, larval settlement was inhibited after a 72 h exposure (30% vs. 47.8% in the control). In each Ca^{2+} treatment (10–25 mM), the percentages of larval settlement and metamorphosis did not significantly differ from the control. Mg^{2+} inhibited settlement and metamorphosis of *M. sallei* larvae at concentrations of 30 and 50 mM. The inhibitive effects on larval settlement and metamorphosis were also observed for NH_4^+ with concentrations above 10 mM. Meanwhile, exposure to NH_4^+ higher than 10 mM caused a significant increase in mortality, indicating that NH_4^+ inhibited *M. sallei* settlement and metamorphosis through acute toxicity.

Larval settlement and metamorphosis responses to the voltage-gated potassium channel inhibitor, TEA, are shown in Figure 4. Compared to the control (FSW containing only 9 mM KCl), treatments of 5×10^{-5} and 10^{-4} M TEA (both mixed with 9 mM KCl) significantly decreased the percentages of larval settlement and metamorphosis, suggesting that the inducing effects of excess K^+ could be inhibited by TEA concentrations above 5×10^{-5} M concentrations.

Larval settlement and metamorphosis in response to neuroactive compounds

The effects of neurotransmitters including GABA, L-DOPA, dopamine and epinephrine on larval

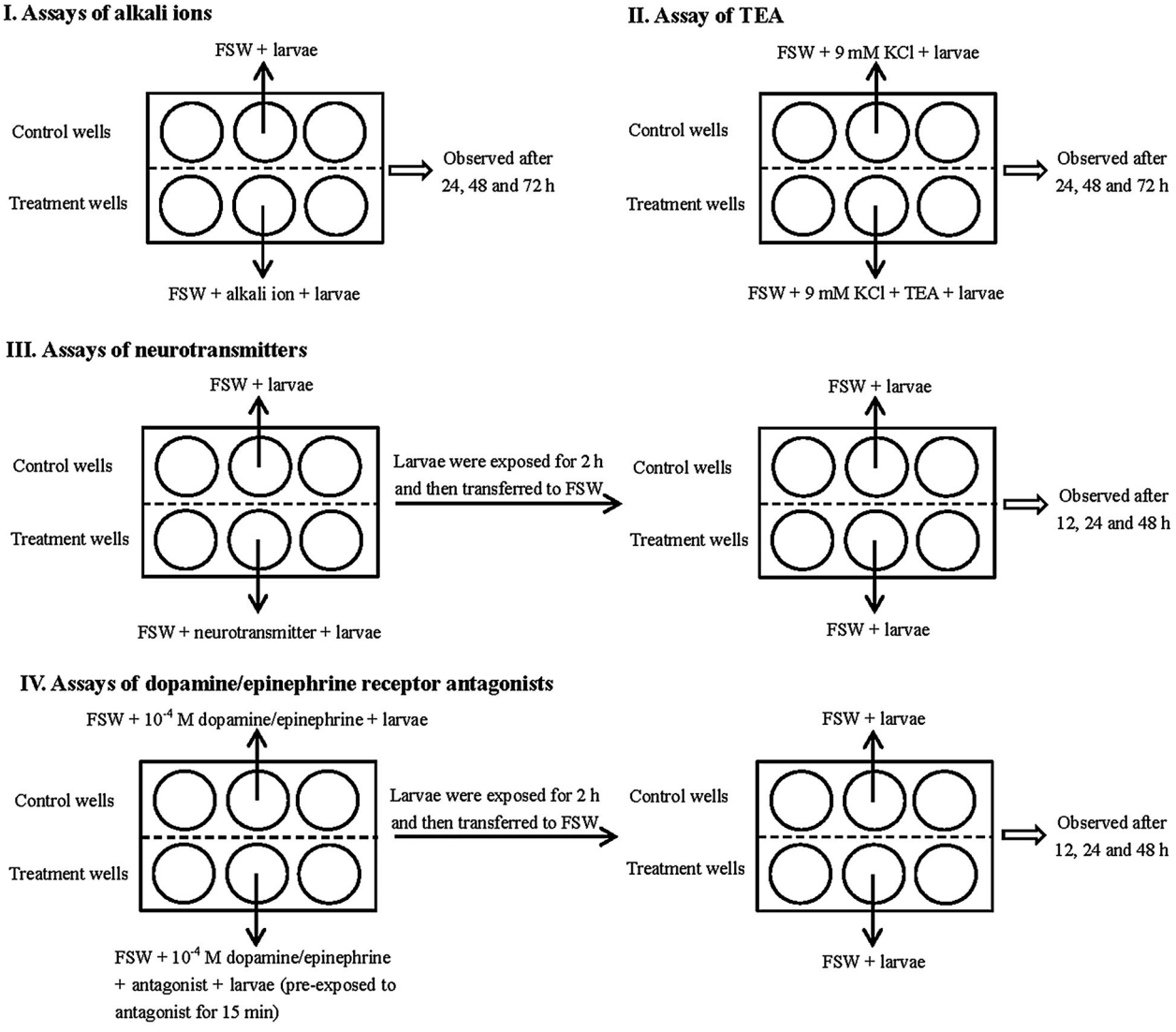

Figure 2. Schematic presentation of experimental design.

settlement and metamorphosis of *Mytilopsis sallei* are shown in Figure 5. When treated with 10^{-6}–5×10^{-5} M GABA, larval settlement and metamorphosis did not differ significantly from the control. However, at a higher concentration of 10^{-4} M, GABA inhibited larval settlement and metamorphosis and displayed lethal toxicity. At 5×10^{-5} and 10^{-4} M L-DOPA, larval settlement and metamorphosis were significantly higher when compared to the control. Furthermore, treatment with 10^{-5} M L-DOPA increased larval settlement when compared to the control at 48 h, suggesting inductive effects of L-DOPA on *M. sallei* settlement and metamorphosis. For dopamine, significant inducing effects on settlement and metamorphosis of *M. sallei* larvae were found at concentrations from 10^{-5} to 10^{-4} M. In the case of epinephrine, treatments at 5×10^{-5} and 10^{-4} M concentrations induced larval settlement and metamorphosis significantly. Moreover, treatments of 5×10^{-6} and 10^{-5} M epinephrine also significantly induced larval metamorphosis at 48 h.

Larval settlement and metamorphosis in response to two dopamine receptor antagonists (SCH23390 and sulpiride) in the presence of 10^{-4} M dopamine are shown in Figure 6. When compared to the control, significantly lower percentages of larval settlement and metamorphosis were observed after treatments with SCH23390 at concentrations ranging from 10^{-5} to 10^{-4} M, indicating that the inducing effects of 10^{-4} M dopamine could be inhibited by SCH23390. In the case of sulpiride, no significant differences in the percentages of settlement and metamorphosis were evident between each treatment with sulpiride and the control, suggesting that sulpiride did not inhibit the inducing effects of dopamine on *M. sallei* settlement and metamorphosis.

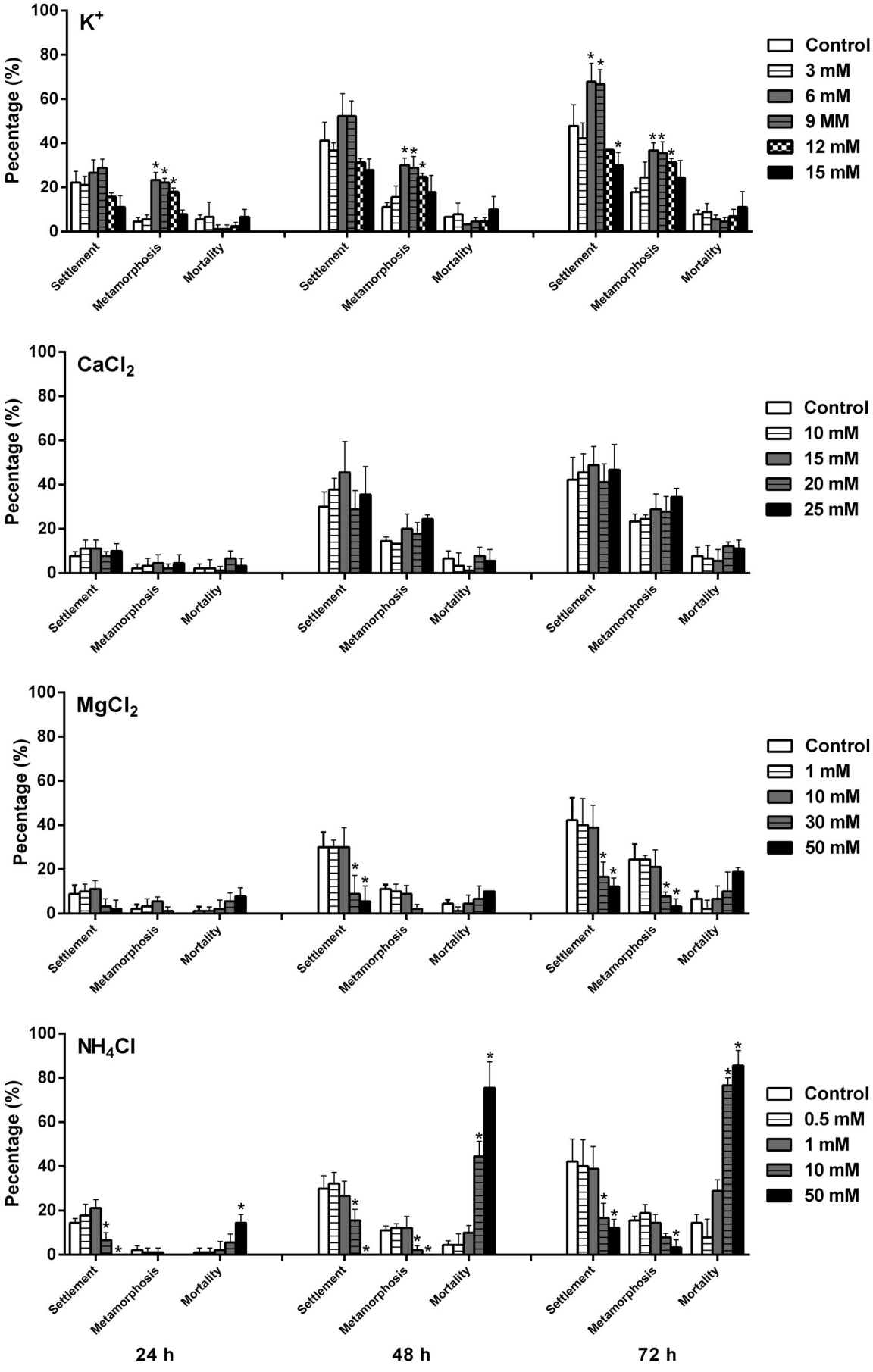

Figure 3. Percentages of settlement, metamorphosis and mortality of *Mytilopsis sallei* larvae after exposure to various concentrations of alkali ions for 24, 48 and 72 h. Control larvae were exposed to filtered seawater. Data are mean values of three replicates with standard deviations indicated by vertical bars. * denotes significant difference from the control ($P < 0.05$, one-way ANOVA).

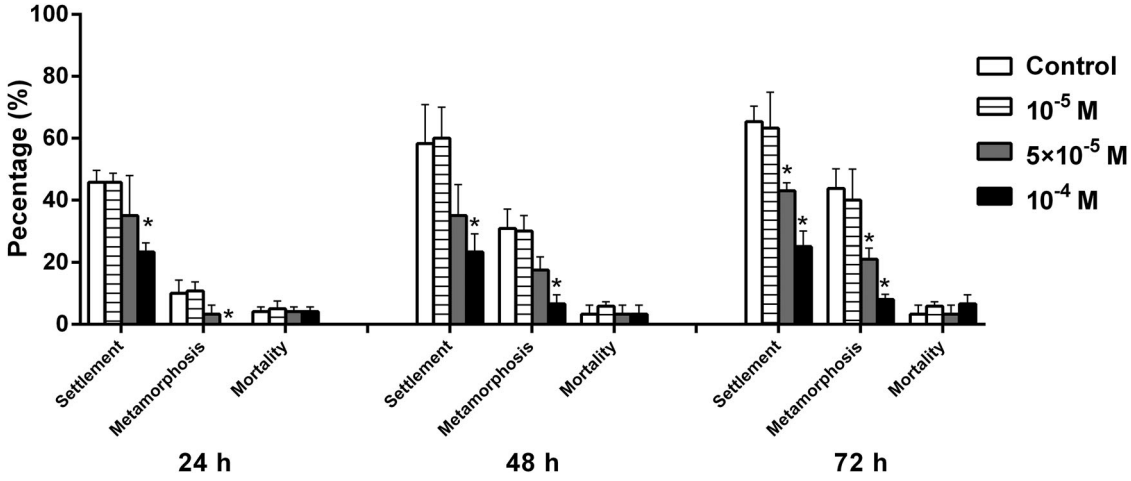

Figure 4. Percentages of settlement, metamorphosis and mortality of *Mytilopsis sallei* larvae after exposure to a mixture of tetraethylammonium (varying concentrations) and KCl (9 mM) for 24, 48 and 72 h. Control larvae were exposed to filtered seawater containing only 9 mM KCl. Data are mean values of three replicates with standard deviations indicated by vertical bars. * denotes significant difference from the control ($P < 0.05$, one-way ANOVA).

Larval settlement and metamorphosis in response to four adrenergic antagonists (chlorpromazine, yohimbine, atenolol and butoxamine) in the presence of 10^{-4} M epinephrine are shown in Figure 7. When treated with chlorpromazine at 10^{-5}–10^{-4} M concentrations, the percentages of settlement and metamorphosis decreased significantly when compared to the control. Furthermore, the treatment of larvae with 5×10^{-6} M chlorpromazine significantly lowered the percentage of settlement when compared to the control at 48 h. It should be noted that when the concentration of chlorpromazine reached above 5×10^{-5} M, acute toxicity was observed on *M. sallei* larvae. It was evident that the inducing effect of 10^{-4} M epinephrine on larval settlement and metamorphosis could be inhibited by chlorpromazine at non-toxic concentrations such as 5×10^{-6} and 10^{-5} M. Treatments with yohimbine, atenolol and butoxamine did not significantly affect larval settlement and metamorphosis when compared to the control, indicating that these three adrenergic antagonists did not inhibit the inducing effects of epinephrine on *M. sallei* settlement and metamorphosis.

Table II. Effects of listed chemical compounds on larval settlement and metamorphosis of *Mytilopsis sallei*.

Chemical compounds[a]	Inductive	Inhibitive	No effect
Alkali ions			
KCl	√		
$CaCl_2$			√
$MgCl_2$		√	
NH_4Cl		√	
Neurotransmitters			
GABA		√	
L-DOPA	√		
Dopamine	√		
Epinephrine	√		
Blockers			
TEA[b]		√	
SCH23390[c]		√	
Sulpiride[c]			√
Chlorpromazine[d]		√	
Yohimbine[d]			√
Atenolol[d]			√
Butoxamine[d]			√

[a]Abbreviations for GABA, L-DOPA, TEA and SCH23390 are described in Table I.
[b]Larvae were exposed to the compound in presence of 9 mM KCl.
[c]Larvae were exposed to the compound in presence of 10^{-4} M dopamine.
[d]Larvae were exposed to the compound in presence of 10^{-4} M epinephrine.

Discussion

Investigations on the effects of pharmacological substances on the settlement and metamorphosis of marine invertebrates are helpful to understand the biochemical pathways involved in these processes (Bonar et al. 1990; Pawlik 1990; Young 2009; Wang et al. 2015). In this study, we investigated the responses of *Mytilopsis sallei* larval settlement and metamorphosis to alkali ions and neuroactive compounds. The effects of these compounds are summarized in Table II. This is the first study to show the responses of larval settlement and metamorphosis of dreissenid mussels to pharmacological compounds.

Potassium has been shown to induce larval settlement and metamorphosis in many marine invertebrates in a dose-dependent and species-specific manner (Yool et al. 1986; Young 2009; Wang et al. 2015). For example, Wang et al. (2015) found that while 10 mM excess K^+ significantly promoted the settlement of oyster, *Crassostrea gigas* (Thunberg,

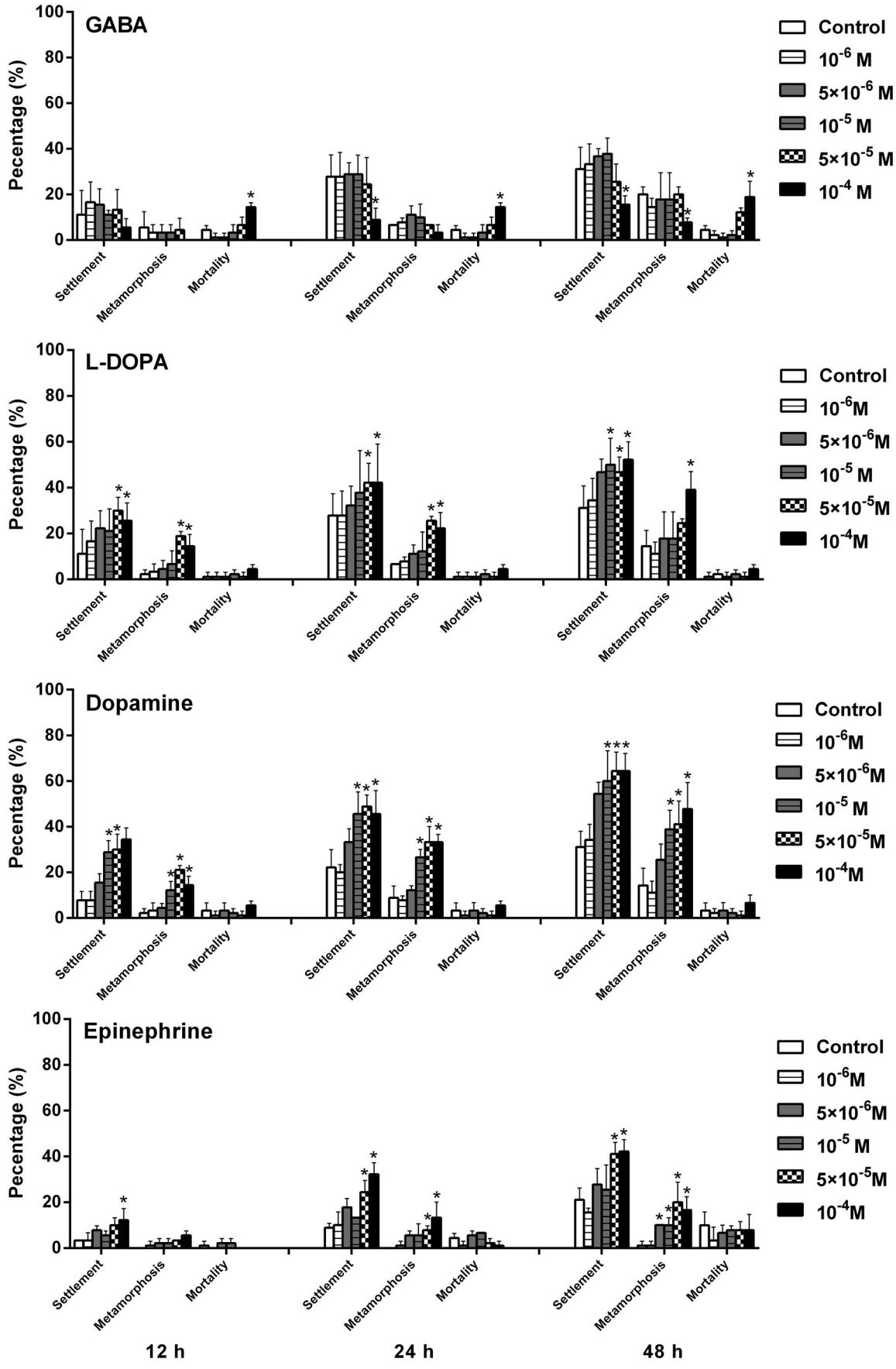

Figure 5. Percentage of settlement, metamorphosis and mortality in *Mytilopsis sallei* larvae after exposure to varying concentrations of neuroactive compounds for 2 h followed by incubation in filtered seawater for 12, 24 and 48 h. Control larvae were exposed to filtered seawater throughout the experiment. Data are mean values of three replicates with standard deviations indicated by vertical bars. * denotes significant difference from the control ($P < 0.05$, one-way ANOVA).

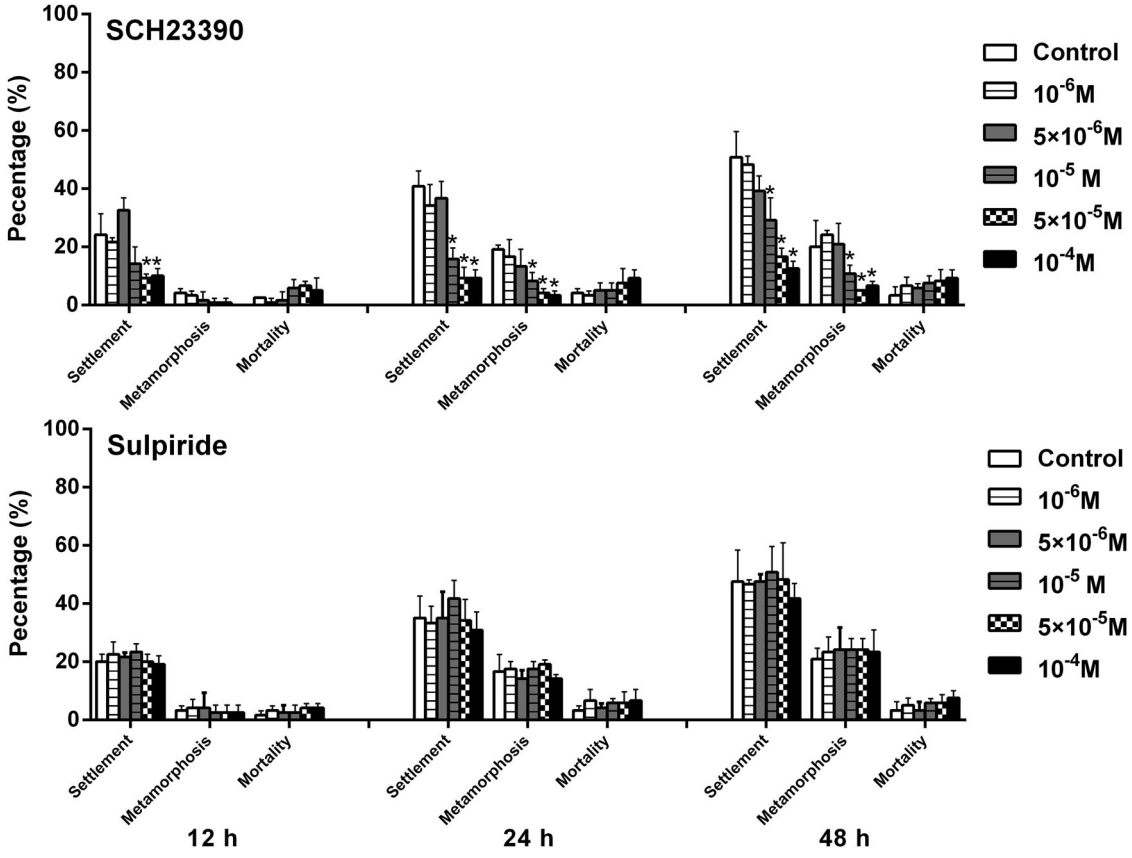

Figure 6. Percentages of settlement, metamorphosis and mortality of *Mytilopsis sallei* larvae after exposure to dopamine receptor antagonists for 15 min, followed by a mixture of antagonists and 10^{-4} M dopamine for 2 h, and incubated in filtered seawater for 12, 24 and 48 h. Control larvae were exposed to 10^{-4} M dopamine for 2 h and then transferred to filtered seawater. Data are mean values of three replicates with standard deviations indicated by vertical bars. * denotes significant difference from the control ($P < 0.05$, one-way ANOVA).

1793), concentrations higher than 15 mM K^+ inhibited larval settlement. Moreover, treatment with 5–15 mM excess K^+ significantly increased larval settlement of the green mussel, *Perna canaliculus* (Gmelin, 1791) (Young 2009), while excess K^+ at concentrations of 5–40 mM had no significant effect on larval settlement in the blue mussel *Mytilus edulis* Linnaeus, 1758 (Eyster & Pechenik 1988; Dobretsov & Qian 2003). In the present study, *M. sallei* larvae were induced to settle by 6–9 mM excess K^+ and to metamorphose by 6–12 mM excess K^+. Potassium has been suggested to induce larval settlement and metamorphosis of marine invertebrates by depolarizing externally accessible and excitable cells involved in the larval perception of inducers (Baloun & Morse 1984; Yool et al. 1986). Furthermore, a few studies reported that TEA significantly inhibited the inducing effects of K^+ on larval settlement and metamorphosis (Ke et al. 1998; Yang et al. 2011; Wang et al. 2015), suggesting that a voltage-gated potassium channel played an important role in the processes. Our results were consistent with these studies and indicated that the voltage-gated potassium channel could be involved in the settlement and metamorphosis of *M. sallei* larvae.

Calcium has also been reported as an inducer of larval settlement and metamorphosis in some invertebrate species (Yool et al. 1986; Zhao et al. 2003; Amador-Cano et al. 2006; Yang et al. 2013). However, we found no significant effects of Ca^{2+} on *M. sallei* larval settlement and metamorphosis, suggesting that calcium may not be involved in the signal transduction pathways of these processes in *M. sallei*. Increasing Ca^{2+} levels also did not induce larval metamorphosis of the bivalves *M. unguiculatus* (Yang et al. 2013) and *Perna viridis* (Linnaeus, 1758) (Ke et al. 1998).

Compared to potassium and calcium, magnesium has received less attention for its effects on the larval settlement and metamorphosis of marine invertebrates. Excess Mg^{2+} showed no inducing effect in the pearl oyster *Pinctada fucata martensii* (Dunker, 1872) (Yu et al. 2008) and the green mussel *P. viridis* (Ke et al. 1998). However, Yu et al. (2007) found that

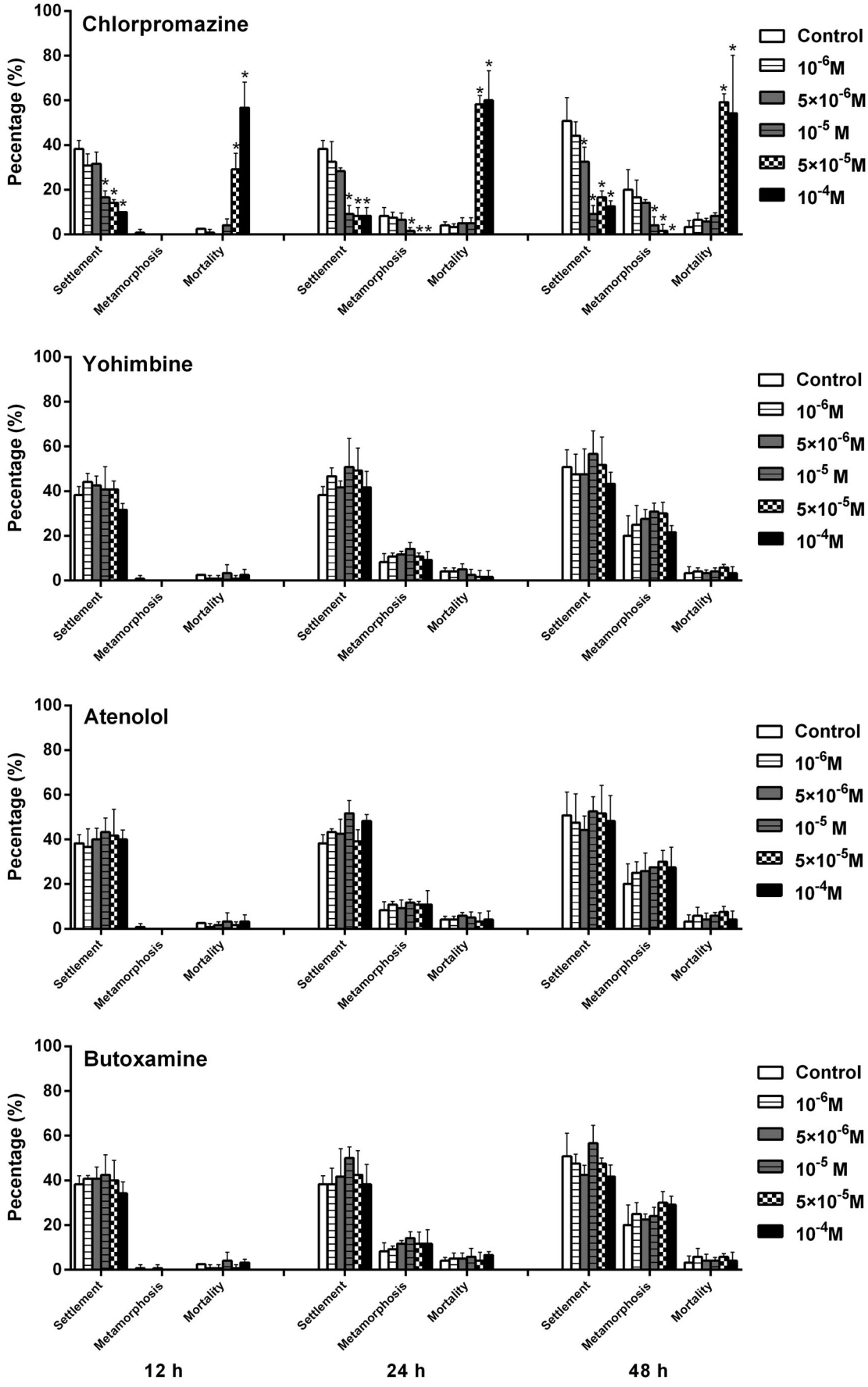

Figure 7. Percentage of settlement, metamorphosis and mortality of *Mytilopsis sallei* larvae after exposure to adrenergic antagonists for 15 min, followed by a mixture of antagonists and 10^{-4} M epinephrine for 2 h, and then incubated in filtered seawater for 12, 24 and 48 h. Control larvae were exposed to 10^{-4} M epinephrine for 2 h and then transferred to filtered seawater. Data are mean values of three replicates with standard deviations indicated by vertical bars. * denotes significant difference from the control ($P < 0.05$, one-way ANOVA).

increasing Mg^{2+} inhibited larval settlement and metamorphosis of the bryozoan *Bugula neritina* (Linnaeus, 1758). In this study, we found that Mg^{2+} had an inhibitory effect on *M. sallei* larvae.

Ammonium was found to be an effective inducer of larval settlement behaviour in the oysters *C. gigas* (Coon et al. 1990) and *C. virginica* (Gmelin, 1791) (Fitt & Coon 1992). Furthermore, Coon et al. (1990) suggested that it was actually NH_3, not NH_4^+, in the solutions that induced settlement behaviour, and NH_3 acted by increasing intracellular pH. However, in the pearl oyster, *P. maxima* (Jameson, 1901) (Zhao et al. 2003), ammonium did not induce larval settlement, which is consistent with our findings for *M. sallei*.

The inducing effects of GABA on the larval settlement and metamorphosis of marine invertebrates was first demonstrated in the gastropod *Haliotis rufescens* Swainson, 1822 (Morse et al. 1979). It was suggested that GABA's inducing effects could most likely be due to its close structural resemblance to a natural inducer, which was isolated from *Lithothamnium* and *Porphyra*, and probably contains a conjugated GABA-like moiety (δ-aminolevulinic acid) (Morse et al. 1984; Morse & Morse 1984; Morse 1985). Thus far, GABA has been reported to induce larval settlement in several bivalve species (García-Lavandeira et al. 2005; Alfaro et al. 2011; Mesías-Gansbiller et al. 2013; Yang et al. 2013). In the present work, GABA did not induce the larval settlement and metamorphosis of *M. sallei*, indicating that it is unlikely to be a natural inducer or functional analogue for this species. Similarly, other mussels, such as *M. edulis* (Dobretsov & Qian 2003) and *P. canaliculus* (Young et al. 2011), also showed no inducing response to GABA.

Many researchers have shown that catecholamines (such as dopamine and epinephrine) and L-DOPA are especially important for modulating the larval settlement and metamorphosis of marine invertebrates, including bivalves (Teh et al. 2012; Yang et al. 2014; Young et al. 2015), bryozoans (Yu et al. 2007) and ascidians (Kimura et al. 2003; Zega et al. 2005). It has been suggested that L-DOPA is one of the intermediates in the biosynthesis of endogenous dopamine, which can be further converted to epinephrine (Young et al. 2015). In addition, Bonar et al. (1990) proposed that exogenous L-DOPA could be decarboxylated to dopamine within the oyster larvae and could act through dopaminergic receptors. Our results showed that L-DOPA, dopamine and epinephrine effectively induced larval settlement and metamorphosis in *M. sallei*, indicating that the epinephrine biosynthesis pathway may be present in this species and may be involved in the regulation of larval settlement and metamorphosis. The endogenous regulation of larval settlement by a catecholaminergic mechanism was also suggested for *P. canaliculus* by Young et al. (2015), who investigated larval settlement responses after exposure to catecholamines and their precursor metabolites. Further evidence for endogenous regulation by catecholamines was provided by Yang et al. (2012), who cloned the adrenergic-like receptor gene (ARcga) from *C. angulata* (Lamarck, 1819), and proposed that ARcga might play a considerable role in signal transduction during larval metamorphosis.

Furthermore, our results showed that the inducing effects of dopamine on *M. sallei* settlement and metamorphosis could be inhibited by SCH23390 (a selective D1 dopamine receptor antagonist), although sulpiride (a selective D2 dopamine receptor antagonist) exhibited no inhibiting activity, suggesting that the D1 dopamine receptor may play an important role in the settlement and metamorphosis of *M. sallei* larvae. Moreover, the inducing effects of epinephrine on *M. sallei* settlement and metamorphosis were found to be inhibited by chlorpromazine (α_1-adrenergic antagonist), but not by the other three adrenergic antagonists tested (yohimbine (α_2-adrenergic antagonist), atenolol (β_1-adrenergic antagonist) and butoxamine (β_2-adrenergic antagonist)). This indicates that an α_1-adrenoceptor may be involved in the larval settlement and metamorphosis of *M. sallei*, these findings being consistent with previous reports on *C. gigas* (Coon & Bonar 1987), *M. galloprovincialis* Lamarck, 1819 (Yang et al. 2011) and *M. unguiculatus* (Yang et al. 2014).

In conclusion, the bioassays of *M. sallei* larval settlement and metamorphosis reported here for the first time showed that larvae exhibited various responses to different pharmacological compounds. Based on the results, we suggest that the voltage-gated potassium channel, D1 dopamine receptor and the α_1-adrenoceptor may be involved in the settlement and metamorphosis of *M. sallei* larvae. The findings of this study provide new insights on the biochemical mechanisms of larval settlement and metamorphosis in dreissenid mussels, which may have useful implications for their control.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Alfaro AC, Young T, Ganesan AM. 2011. Regulatory effects of mussel (*Aulacomya maoriana* Iredale 1915) larval settlement by neuroactive compounds, amino acids and bacterial biofilms. Aquaculture 322–323:158–68. doi:10.1016/j.aquaculture.2011.08.038

Almeida JR, Vasconcelos V. 2015. Natural antifouling compounds: effectiveness in preventing invertebrate settlement and adhesion. Biotechnology Advances 33:343–57. doi:10.1016/j.biotechadv.2015.01.013

Amador-Cano G, Carpizo-ltuarte E, Cristino-Jorge D. 2006. Role of protein kinase C, G-protein coupled receptors, and calcium flux during metamorphosis of the sea urchin *Strongylocentrotus purpuratus*. The Biological Bulletin 210:121–31. doi:10.2307/4134601

Baloun AJ, Morse DE. 1984. Ionic control of settlement and metamorphosis in larval *Haliotis rufescens* (Gastropoda). The Biological Bulletin 167:124–38. doi:10.2307/1541342

Beiras R, Widdows J. 1995. Induction of metamorphosis in larvae of the oyster *Crassostrea gigas* using neuroactive compounds. Marine Biology 123:327–34. doi:10.1007/BF00353624

Bonar DB, Coon SL, Walch M, Weiner RM, Fitt W. 1990. Control of oyster settlement and metamorphosis by endogenous and exogenous chemical cues. Bulletin of Marine Science 46:484–98.

Cai LZ, Gao Y, Liu WM, Lin XC, Zhou XP, Jin L, et al. 2006. Effect of exotic species *Mytilopsis sallei* on macrozoobenthos in the Maluan Bay, Xiamen in China. Acta Oceanologica Sinica 28:83–89. (in Chinese)

Cai LZ, Hwang JS, Dahms HU, Fu SJ, Zhuo Y, Guo T. 2014. Effect of the invasive bivalve *Mytilopsis sallei* on the macrofaunal fouling community and the environment of Yundang Lagoon, Xiamen, China. Hydrobiologia 741:101–11. doi:10.1007/s10750-014-2012-4

Chu KH, Tam PF, Fung CH, Chen QC. 1997. A biological survey of ballast water in container ships entering Hong Kong. Hydrobiologia 352:201–06. doi:10.1023/A:1003067105577

Clare AS. 2011. Toward a characterization of the chemical cue to barnacle gregariousness. In: Breithaupt T, Thiel M, editors. Chemical Communication in Crustaceans. New York: Springer, p 431–50.

Connell JH. 1985. The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. Journal of Experimental Marine Biology and Ecology 93:11–45. doi:10.1016/0022-0981(85)90146-7

Coon SL, Bonar DB. 1987. Pharmacological evidence that alpha$_1$-adrenoceptors mediate metamorphosis of the Pacific oyster, *Crassostrea gigas*. Neuroscience 23:1169–74. doi:10.1016/0306-4522(87)90190-4

Coon SL, Walch M, Fitt WK, Weiner RM, Bonar DB. 1990. Ammonia induces settlement behavior in oyster larvae. The Biological Bulletin 179:297–303. doi:10.2307/1542321

Crisp DJ. 1984. Overview of research in marine invertebrate larvae 1940–1980. In: Costlow JD, Tipper RC, editors. Marine Biodeterioration: An Interdispciplinary Study. Annapolis, MD: Naval Institute Press, p 103–26.

Dobretsov SV, Qian PY. 2003. Pharmacological induction of larval settlement and metamorphosis in the blue mussel *Mytilus edulis* L. Biofouling 19:57–63. doi:10.1080/0892701021000060860

Dreanno C, Matsumura K, Dohmae N, Takio K, Hirota H, Kirby RR, Clare AS. 2006. An α_2-macroglobulin-like protein is the cue to gregarious settlement of the barnacle *Balanus amphitrite*. Proceedings of the National Academy of Sciences 103:14396–401. doi:10.1073/pnas.0602763103

Eyster LS, Pechenik JA. 1988. Attachment of *Mytilus edulis* L. larvae on algal and byssal filaments is enhanced by water agitation. Journal of Experimental Marine Biology and Ecology 114:99–110. doi:10.1016/0022-0981(88)90131-1

Fitt WK, Coon SL. 1992. Evidence for ammonia as a natural cue for recruitment of oyster larvae to oyster beds in a Georgia salt marsh. The Biological Bulletin 182:401–08. doi:10.2307/1542259

García-Lavandeira M, Silva A, Abad M, Pazos AJ, Sánchez JL, Pérez-Parallé ML. 2005. Effects of GABA and epinephrine on the settlement and metamorphosis of the larvae of four species of bivalve molluscs. Journal of Experimental Marine Biology and Ecology 316:149–56. doi:10.1016/j.jembe.2004.10.011

Gohad NV, Aldred N, Orihuela B, Clare AS, Rittschof D, Mount AS. 2012. Observations on the settlement and cementation of barnacle (*Balanus amphitrite*) cyprid larvae after artificial exposure to noradrenaline and the locations of adrenergic-like receptors. Journal of Experimental Marine Biology and Ecology 416–417:153–61. doi:10.1016/j.jembe.2012.02.013

Grant MN, Meritt DW, Kimmel DG. 2013. Chemical induction of settlement behavior in larvae of the eastern oyster *Crassostrea virginica* (Gmelin). Aquaculture 402–403:84–91. doi:10.1016/j.aquaculture.2013.03.031

Hadfield MG, Paul VJ. 2001. Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. In: McClintock JB, Baker BJ, editors. Marine Chemical Ecology. Boca Raton, FL : CRC Press, p 431–61.

He J, Qi JF, Feng DQ, Ke CH. 2015. Embryonic and larval development of the invasive biofouler *Mytilopsis sallei* (Récluz, 1849) (Bivalvia: Dreissenidae). Journal of Molluscan Studies 82(1):23–30. doi:10.1093/mollus/eyv026

Higgins SN, Zanden MJ. 2010. What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. Ecological Monographs 80:179–96. doi:10.1890/09-1249.1

Jin C, Qiu J, Yu S, Miao L, Zhou X. 2014. Histamine promotes the larval metamorphic competence of barnacle *Amphibalanus amphitrite*. Marine Biology Research 10:799–806. doi:10.1080/17451000.2013.863352

Jin T, Qian PY. 2004. Effect of amino acids on larval metamorphosis of the polychaete *Hydroides elegans*. Marine Ecology Progress Series 267:209–18. doi:10.3354/meps267209

Johnson LE, Carlton JT. 1996. Post-establishment spread in large-scale invasions: dispersal mechanisms of the zebra mussel *Dreissena polymorpha*. Ecology 77:1686–90. doi:10.2307/2265774

Ke CH, Li SJ, Li FX, Zheng Z. 1994. Induction of catecholamines and its analogs on settlement and metamorphasis of the green mussel, *Perna viridis* (Linnaeus). Journal of Xiamen University 34:975–81. (in Chinese)

Ke CH, Li SJ, Li FX, Zheng Z. 1998. Ionic control of attachment and metamorphosis in the green mussel *Perna viridis* (Linnaeus) larvae. Oceanologia et Limnologia Sinica 29:128–34. (in Chinese)

Kennedy VS. 2011. The invasive dark falsemussel *Mytilopsis leucophaeata* (Bivalvia: Dreissenidae): a literature review. Aquatic Ecology 45:163–83. doi:10.1007/s10452-010-9344-6

Kimura Y, Yoshida M, Morisawa M. 2003. Interaction between noradrenaline or adrenaline and the β_1-adrenergic receptor in the nervous system triggers early metamorphosis of larvae in the ascidian, *Ciona savignyi*. Developmental Biology 258:129–40. doi:10.1016/S0012-1606(03)00118-0

Liao CM, Ju YR, Chio CP, Chen WY. 2010. Risk-based probabilistic approach to assess the impact of false mussel invasions on farmed hard clams. Risk Analysis 30:310–23. doi:10.1111/j.1539-6924.2009.01315.x

Matthews J, Van der Velde G, De Vaate AB, Collas FPL, Koopman KR, Leuven RSEW. 2014. Rapid range expansion of the invasive quagga mussel in relation to zebra mussel presence in The Netherlands and Western Europe. Biological Invasions 16:23–42. doi:10.1007/s10530-013-0498-8

Mesías-Gansbiller C, Silva A, Maneiro V, Pazos A, Sánchez JL, Pérez-Parallé ML. 2013. Effects of chemical cues on larval settlement of the flat oyster (*Ostrea edulis* L.): A hatchery approach. Aquaculture 376–379:85–89. doi:10.1016/j.aquaculture.2012.11.022

Morse ANC, Morse DE. 1984. GABA-mimetic molecules from *Porphyra* (Rhodophyta) induce metamorphosis of *Haliotis* (Gastropoda) larvae. Hydrobiologia 116–116:155–58. doi:10.1007/BF00027656

Morse ANC, Froyd CA, Morse DE. 1984. Molecules from cyanobacteria and red algae that induce larval settlement and metamorphosis in the mollusc *Haliotis rufescens*. Marine Biology 81:293–98. doi:10.1007/BF00393223

Morse DE. 1985. Neurotransmitter-mimetic inducers of larval settlement and metamorphosis. Bulletin of Marine Science 37:697–706.

Morse DE, Hooker N, Duncan H, Jensen L. 1979. γ-Aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. Science 204:407–10. doi:10.1126/science.204.4391.407

Morton B. 1981. The biology and functional morphology of *Mytilopsis sallei* (Recluz) (Bivalvia: Dreissenacea) fouling Visakhapatnam Harbour, Andhra Pradesh, India. Journal of Molluscan Studies 47:25–42.

O'Connor S, Moltschaniwskyj N, O'Connor W. 2009. Use of neuroactive catecholamines to chemically induce metamorphosis of hatchery-reared flat oyster, *Ostrea angasi*, larvae. Aquaculture Research 40:1567–77. doi:10.1111/j.1365-2109.2009.02203.x

Pawlik JR. 1986. Chemical induction of larval settlement and metamorphosis in the reef-building tube worm *Phragnmtopoma californica* (Sabellariidae: Polychaeta). Marine Biology 91:59–68. doi:10.1007/BF00397571

Pawlik JR. 1990. Natural and artificial induction of metamorphosis of *Phragmatopoma lapidosa californica* (Polychaeta: Sabellariidae), with a critical look at the effects of bioactive compounds on marine invertebrate larvae. Bulletin of Marine Science 46:512–36.

Pawlik JR. 1992. Chemical ecology of the settlement of benthic marine invertebrates. Oceanography and Marine Biology: An Annual Review 30:273–335.

Prendergast GS. 2010. Settlement and behaviour of marine fouling organisms. In: Dürr S, Thomason JC, editors. Biofouling. Chichester, UK: Wiley-Blackwell, p 30–59.

Qian PY, Chen L, Xu Y. 2013. Mini-review: Molecular mechanisms of antifouling compounds. Biofouling 29:381–400. doi:10.1080/08927014.2013.776546

Swanson RL, Williamson JE, De Nys R, Kumar N, Bucknall MP, Steinberg PD. 2004. Induction of settlement of larvae of the sea urchin *Holopneustes purpurascens* by histamine from a host alga. The Biological Bulletin 206:161–72. doi:10.2307/1543640

Tan KS, Morton B. 2006. The invasive Caribbean bivalve *Mytilopsis sallei* (Dreissenidae) introduced to Singapore and Johor Bahru, Malaysia. Raffles Bulletin of Zoology 54:429–34.

Tebben J, Tapiolas DM, Motti CA, Abrego D, Negri AP, Blackall LL, et al. 2011. Induction of larval metamorphosis of the coral *Acropora millepora* by tetrabromopyrrole isolated from a *Pseudoalteromonas* bacterium. PLoS One 6: e19082. 8 pages. doi:10.1371/journal.pone.0019082

Teh CP, Zulfigar Y, Tan SH. 2012. Epinephrine and L-DOPA promote larval settlement and metamorphosis of the tropical oyster, *Crassostrea iredalei* (Faustino, 1932): an oyster hatchery perspective. Aquaculture 338–341:260–63. doi:10.1016/j.aquaculture.2012.01.014

Tsukamoto S, Kato H, Hirota H, Fusetani N. 1999. Lumichrome. A larval metamorphosis-inducing substance in the ascidian Halocynthia roretzi. European Journal of Biochemistry 264:785–89. doi:10.1046/j.1432-1327.1999.00642.x

Wang J, Wu C, Xu C, Yu W, Li Z, Li Y, et al. 2015. Voltage-gated potassium ion channel may play a major role in the settlement of Pacific oyster (*Crassostrea gigas*) larvae. Aquaculture 442:48–50. doi:10.1016/j.aquaculture.2015.02.033

Wong YT, Meier R, Tan KS. 2011. High haplotype variability in established Asian populations of the invasive Caribbean bivalve *Mytilopsis sallei* (Dreissenidae). Biological Invasions 13:341–48. doi:10.1007/s10530-010-9825-5

Yang B, Qin J, Shi B, Han G, Chen J, Huang H, He C. 2012. Molecular characterization and functional analysis of adrenergic like receptor during larval metamorphosis in *Crassostrea angulata*. Aquaculture 366–367:54–61. doi:10.1016/j.aquaculture.2012.08.040

Yang JL, Satuito GC, Bao WY, Kitamura H. 2008. Induction of metamorphosis of pediveliger larvae of the mussel *Mytilus galloprovincialis* Lamarck, 1819 using neuroactive compounds, KCl, NH_4Cl and organic solvents. Biofouling 24:461–70. doi:10.1080/08927010802340309

Yang JL, Li YF, Bao WY, Satuito CG, Kitamura H. 2011. Larval metamorphosis of the mussel *Mytilus galloprovincialis* Lamarck, 1819 in response to neurotransmitter blockers and tetraethylammonium. Biofouling 27:193–99. doi:10.1080/08927014.2011.553717

Yang JL, Li SH, Li YF, Liu ZW, Liang X, Bao WY, Li JL. 2013. Effects of neuroactive compounds, ions and organic solvents on larval metamorphosis of the mussel *Mytilus coruscus*. Aquaculture 396–399:106–12. doi:10.1016/j.aquaculture.2013.02.039

Yang JL, Li WS, Liang X, Li YF, Chen YR, Bao WY, Li JL. 2014. Effects of adrenoceptor compounds on larval metamorphosis of the mussel *Mytilus coruscus*. Aquaculture 426–427:282–87. doi:10.1016/j.aquaculture.2014.02.019

Yool AJ, Grau SM, Hadfield MG, Jensen RA, Markell DA, Morse DE. 1986. Excess potassium induces larval metamorphosis in four marine invertebrate species. The Biological Bulletin 170:255–66. doi:10.2307/1541807

Young T. 2009. Pharmalogical Induction of Larval Settlement in the New Zealand Mussel *Perna canaliculus*. Master's Thesis. Auckland University of Technology, Auckland: Applied Science in Marine & Environmental Sciences. 348 pages.

Young T, Alfaro AC, Robertson J. 2011. Effect of neuroactive compounds on the settlement of mussel (*Perna canaliculus*) larvae. Aquaculture 319:277–83. doi:10.1016/j.aquaculture.2011.06.050

Young T, Alfaro AC, Sánchez-Lazo C, Robertson J. 2015. Putative involvement of adrenergic receptors in regulation of mussel (*Perna canaliculus*) larval settlement. Marine Biology Research 11:655–65. doi:10.1080/17451000.2014.979833

Yu X, Yan Y, Gu JD. 2007. Attachment of the biofouling bryozoan *Bugula neritina* larvae affected by inorganic and organic chemical cues. International Biodeterioration & Biodegradation 60: 81–89. doi:10.1016/j.ibiod.2006.12.003

Yu X, He W, Gu JD, He M, Yan Y. 2008. The effect of chemical cues on settlement of pearl oyster *Pinctada fucata martensii* (Dunker) larvae. Aquaculture 277:83–91. doi:10.1016/j.aquaculture.2008.02.010

Yvin JC, Chevolot L, Chevolot-Magueur AM, Cochard JC. 1985. First isolation of jacaraone from an alga, *Delesseria sanguinea*. A metamorphosis inducer of *Pecten* larvae. Journal of Natural Products 48:814–81. doi:10.1021/np50041a108

Zega G, Pennati R, Groppelli S, Sotgia C, De Bernardi F. 2005. Dopamine and serotonin modulate the onset of metamorphosis in the ascidian *Phallusia mammillata*. Developmental Biology 282:246–56. doi:10.1016/j.ydbio.2005.03.012

Zhao B, Zhang S, Qian PY. 2003. Larval settlement of the silver- or goldlip pearl oyster *Pinctada maxima* (Jameson) in response to natural biofilms and chemical cues. Aquaculture 220:883–901. doi:10.1016/S0044-8486(02)00567-7