



ORIGINAL ARTICLE

Putative involvement of adrenergic receptors in regulation of mussel (*Perna canaliculus*) larval settlement

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Abstract

Settlement responses were investigated for mussel (*Perna canaliculus*) larvae after exposure to catecholamines and their precursor metabolites. Settlement and mortality assays were conducted in Petri plates with chemical treatments (L-phenylalanine, L-tyrosine, L-DOPA, dopamine hydrochloride and epinephrine at various concentrations) and controls. The proteinogenic amino acids L-phenylalanine and L-tyrosine both were effective inducers (~65%) of larval settlement at 10^{-5} mol L⁻¹ compared with controls (4%). Exposure of larvae to L-DOPA, dopamine and epinephrine resulted in maximum settlement induction (50, 60 and 51%, respectively) at 10^{-5} mol L⁻¹. Larval mortalities were low (comparable to controls) across all concentrations of L-phenylalanine and L-tyrosine treatments whereas high mortalities (>60%) were observed for L-DOPA, dopamine and epinephrine at concentrations $\geq 10^{-4}$ mol L⁻¹. Our results indicate that *P. canaliculus* larval settlement is under endogenous regulation by a catecholaminergic mechanism. Furthermore, the inductive effects of all tested metabolites in the epinephrine biosynthesis pathway point to a putative involvement of adrenergic-type receptors in regulation of larval settlement in this mussel species.

Key words: Catecholamines, dopamine, epinephrine, green-lipped mussels, larval settlement, *Perna canaliculus*

Introduction

The distributions of marine invertebrates at various spatial scales depend on their developmental mode and pre-metamorphic behaviour (Foggo et al. 2007). Most of these organisms develop in the plankton for varying durations depending on the species, which predetermines their maximum larval dispersal range. While free-swimming larvae are directly transported by ocean currents and near-surface wind forces (Cowen & Sponaugle 2009), environmental factors regulating their behaviour also have a significant influence on the time spent in the planktonic phase e.g. food availability and quality, salinity, temperature and season (O'Connor et al. 2007; Gebauer et al. 2010). Once larvae reach a critical stage in their development, they become competent to metamorphose into their adult forms. In most cases, this change

in body plan is preceded by movement of the organism from pelagic to benthic environments where larvae search for a suitable attachment substrate. This pre-metamorphic process is termed larval settlement and is mediated, at least partially, by chemoreception of environmental cues (Hadfield 2011). To gain insight into the endogenous signalling pathways involved in larval settlement and metamorphosis, pharmacologically active compounds can be applied in the laboratory to identify their regulatory effects.

Catecholamines (dopamine, norepinephrine and epinephrine) are involved in regulation of various cellular processes and behaviours in vertebrate and invertebrate systems. Within molluscan taxa, these neurotransmitters and hormones are involved in modulation of muscular and ciliary activities and have been implicated in the control of a wide range of critical functional parameters, including early

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ontogenic behaviour (Croll & Dickinson 2004), larval metamorphosis (Yang et al. 2014), sensory development (Wyeth & Croll 2011), cardiac regulation (Kodirov 2011), feeding behaviour (Díaz-Ríos & Miller 2005), reward-seeking behaviour (Barron et al. 2010), respiration (Tsyganov et al. 2004), waste removal (Carroll & Catapane 2007), immune response (Zhou et al. 2011), locomotion (Filla et al. 2009) and reproduction (López-Sánchez et al. 2009). Dopamine, biosynthesized from the essential amino acid L-phenylalanine *via* L-tyrosine and L-β -3, 4-dihydroxyphenylalanine (L-DOPA) amino acid intermediates, can be converted to epinephrine *via* norepinephrine. While L-phenylalanine and L-tyrosine are usually assimilated through dietary means, some marine invertebrates have demonstrated an ability to take up free dissolved amino acids from seawater as an alternative or supplementary route (Söylemez

et al. 2010; Leroy et al. 2012). In molluscs, endogenous levels of catecholamines and/or expression of their receptors vary depending on developmental stage and age (Kreiling et al. 2001), season or reproductive condition (López-Sánchez et al. 2009) and exposure to environmental stressors, such as air exposure, mechanical agitation, temperature and salinity (Chen et al. 2008; Qu et al. 2009). Thus, catecholamine metabolism is an important pathway linking a wide array of molluscan behaviours and their regulation by external stimuli.

Exogenous treatment of marine bivalves with catecholamines and/or their precursor metabolites has resulted in the induction of larval settlement and/or metamorphosis for a wide range of species, including representatives of scallops (Pectinoidea), true oysters (Ostreoida), clams (Veneroida) and mussels (Mytiloidea) (reviewed in Table I). Conversely, L-DOPA,

Table I. Larval responses of bivalve molluscs exposed to L-DOPA (DOP), dopamine (DA), norepinephrine (NE) and epinephrine (E) where: *s* = settlement, *m* = metamorphosis, 0 = no effect, 1 = inductive response. Missing data are represented by a hyphen.

Group	Species	DOP	DA	NE	E	References
Mussels	<i>Perna canaliculus</i>	1s	1s	–	1s	Present study
	<i>Aulacomya maoriana</i>	–	1s	–	1s	Alfaro et al. (2011)
	<i>Mytilus edulis</i>	1s	–	–	–	Dobretsov & Qian (2003)
	<i>Mytilus coruscus</i>	–	1m	–	1m	Yang et al. (2013, 2014)
	<i>Mytilus galloprovincialis</i>	1m	–	1m	1s/1m	Satuito et al. (1999, 2005); García-Lavandeira et al. (2005); Yang et al. (2008); Sánchez-Lazo et al. (2012)
Pearl oysters	<i>Pinctada fucata martensii</i> *	0s	0s	–	–	Yu et al. (2008)
	<i>Pinctada margaritifera</i>	–	–	0s	0s	Doroudi & Southgate (2002)
	<i>Pinctada maxima</i>	0s	0s	–	–	Zhao et al. (2003)
True oysters	<i>Saccostrea glomerata</i>	–	–	–	1s/1m	O'Connor et al. (2008)
	<i>Ostrea angasi</i>	0m	–	–	1m	O'Connor et al. (2009)
	<i>Ostrea edulis</i>	–	–	–	1s/1m	García-Lavandeira et al. (2005)
	<i>Crassostrea virginica</i>	1s	–	0s	0s/1m	Coon et al. (1986); Zimmer-Faust & Tamburri (1994); Grant (2009)
	<i>Crassostrea madrasensis</i>	1s/1m	0s/0m	0s/1m	0s/1m	Murthy et al. (1999)
	<i>Crassostrea belcheri</i>	1s/1m	–	1m	1m	Tan & Wong (1995); Teh et al. (2010)
	<i>Crassostrea iredalei</i> *	1s	–	1s	1s	Teh et al. (2011); Teh et al. (2012)
	<i>Crassostrea brasiliiana</i>	–	1s	1s	1s/1m	Silveira et al. (2011); Alfaro et al. (unpublished data)
	<i>Crassostrea gigas</i>	1s/1m	0–1s†	0s/1m	0s/1m	Coon et al. (1985); Coon et al. (1986); Bonar et al. (1990); Beiras & Widdows (1995); Nicolas et al. (1998)
	Scallops	<i>Chlamys hastata</i>	0m	–	–	–
<i>Chlamys varia</i> *		–	–	–	1s	Mesias-Gansbiller et al. (2008)
<i>Pecten maximus</i>		1m	–	–	1m	Cochard et al. (1989); Nicolas et al. (1996, 1998); Chevolut et al. (1991)
<i>Patinopecten yessoensis</i>		–	–	–	1m	Kingzett et al. (1990)
<i>Argopecten irradians</i>		1m	–	1m	1m	Liu et al. (1998)
Clams	<i>Argopecten purpuratus</i>	–	–	–	1s/1m	Martinez et al. (1999)
	<i>Ruditapes philippinarum</i> *	0m	0m	0m	0s/0–1m	Urrutia et al. (2004); García-Lavandeira et al. (2005); Sumin et al. (2006)
	<i>Venerupis pullastra</i> *	–	–	–	1s/1m	García-Lavandeira et al. (2005)
	<i>Meretrix meretrix</i>	–	–	1m	1m	Wang et al. (2006)
	<i>Lutraria philippinarum</i> *	1s	1s	–	1s	Alfaro et al. (unpublished data)
	<i>Coelomactra antiquata</i> *	1m	–	–	1m	Gao & Lui (2006)

*Species names are listed as described by the referenced literature. However, current taxonomic classifications may differ.

†Settlement behaviour was induced by a selective agonist (SKF 82,526) of D1 dopamine receptors.

dopamine and/or epinephrine do not induce larval settlement in pearl oysters (Pterioida). Catecholamines also have little or no capacity to induce settlement/metamorphosis in the clam *Ruditapes philippinarum* (Adams & Reeve, 1850). Furthermore, for some clades (e.g. the true oysters), settlement and metamorphosis appear to be under differential regulation by dopaminergic and adrenergic mechanisms, respectively. These species-specific responses imply differences in the biochemical mechanisms involved.

The New Zealand green-lipped mussel, *Perna canaliculus* (Gmelin, 1791), is a commercially important aquaculture species, and is farmed extensively for export markets (traded as Greenshell™ mussel). Over the past four decades, substantial research has been conducted on its adult biology, including spatial and temporal population distributions (Paine 1971; Alfaro 2006a; Alfaro et al. 2008), connectivity vectors (Alfaro & Jeffs 2003; Alfaro et al. 2004; Alfaro et al. 2010), habitat preferences and community structure (Paine 1971; Alfaro 2006a; Alfaro et al. 2008), reproductive cycle (Hickman & Illingworth 1980; Alfaro et al. 2001; Alfaro et al. 2003), diet and energetics (Gardner 2002; Alfaro 2006b; Safi & Hayden 2010), behaviour (Kennedy 1976, 1984) and genetics (Gardner et al. 1996; Apte & Gardner 2002; Wei et al. 2013). However, until recently, knowledge of the larval biology of this species was lacking. Partially driven by considerable interest in aquaculture development, current attention is being drawn towards the early life cycle physiology and behaviour of this important commercial species. Recent investigations on the larval settlement process and substrate preferences have identified a variety of exogenous stimuli that have the ability to regulate settlement behaviour. For example, crude chemical fractionates extracted from specific seaweeds with which the juvenile mussels are associated (Alfaro et al. 2006; Gribben et al. 2011), and from marine bacterial biofilms (Ganesan et al. 2010, 2012), have been shown to have settlement-inducing and inhibitory effects. To gain insight into the endogenous control of settlement, we previously investigated effects of the neuroactive compounds γ -amino butyric acid, acetylcholine, atropine and potassium ions on larval settlement of this species (Young et al. 2011). Our results indicate that nicotinic-type acetylcholine receptors are involved in regulation of the settlement process. To further enhance our understanding of endogenous control, we report on the effects of catecholamines and their precursor metabolites on the larval settlement of *P. canaliculus*.

Materials and methods

Chemical treatments

Several compounds involved in epinephrine biosynthesis were tested for their abilities to induce settlement of *Perna canaliculus* larvae. These chemicals included L-phenylalanine (AppliChem), L-tyrosine (AppliChem), L-DOPA (Sigma-Aldrich), dopamine hydrochloride (Sigma-Aldrich) and epinephrine (Sigma-Aldrich). Stock solutions (10 \times concentrates) of each treatment level were prepared by dissolving compounds in 0.45 μ m filtered seawater (FSW) immediately prior to settlement assays. These concentrations were chosen based on a pilot study and our experience with other mussel species (Alfaro et al. 2011; Sánchez-Lazo et al. 2012).

Settlement assays

Settlement-competent veliger larvae (19–23 days post-fertilization) were obtained from Cawthron Institute and Shellfish Production and Technology New Zealand Limited (SPATnz), both in Nelson, New Zealand. The larvae were transported in moist and cold containers to the Auckland University of Technology (AUT) laboratory, Auckland, New Zealand. Upon arrival, the larvae were transferred into a 2 L beaker containing 1 L of FSW. After 30–60 min, healthy swimming larvae were decanted into another beaker, and topped up with FSW to make up a concentration of 20–30 larvae mL⁻¹. Settlement assays were conducted in sterile polystyrene Petri plates (60 \times 14 mm), with 10 replicates per treatment at 17 \pm 1 $^{\circ}$ C under ambient light conditions. Chemical treatment assays consisted of 8 mL FSW, 1 mL larval solution (i.e. 20–30 larvae per Petri plate) and 1 mL concentrated (10 \times) treatment solution. Due to limited larval availability, several experiments were conducted to test the compounds for settlement-inducing ability with mussels from different larval batches. Thus, to standardize the data across experiments, a new set of controls (9 mL FSW and 1 mL larval solution) was used for each batch cohort. Larval settlement and mortality were recorded for all plates after 48 hours, as is optimal for this species (Young et al. 2011; Ganesan et al. 2012). Under a dissecting microscope at 20–45 \times magnification, a 200 μ L displacement pipette was depressed and brought within close proximity (0.5–1.5 mm) to each larva, and gentle suction was applied. Individuals that maintained firm attachment to the substratum were considered settled, and those moving freely with no resistance were considered unsettled. This technique provides a reliable and reproducible way to identify attached/unattached

larvae and is well-established in the literature (Ganesan et al. 2010, 2012; Alfaro et al. 2011; Young et al. 2011; Sánchez-Lazo et al. 2012; Wilkens et al. 2012; McDonald et al. 2014). Furthermore, we have found this method to be consistently more reliable than other methods (e.g. decanting the medium or mechanical agitation of the substrate) for larval settlement assessment. Percentage settlement was calculated as the proportion of settled larvae from the number initially placed in each plate. In many cases, settlement could be detected visually by the presence of thin transparent mucous-like threads, but settlement was always verified by suction.

Mortality assays

To determine acute toxicity effects, mortalities were recorded within the settlement assays under a stereo microscope at 20–45× magnification. Larvae that showed signs of movement of the velum, foot or gut were considered alive. Since live larvae often were inanimate for periods of more than 15 min, neutral red (a vital stain) was used to corroborate mortality detection (Zetsche & Meysman 2012). A 120 ppm solution of neutral red was prepared in FSW and diluted in the experimental medium to give a final stain concentration of 20 ppm. After 30 min, larvae were again viewed at 20× magnification under a stereo microscope. Larvae that did not incorporate the stain into their tissues were considered dead. Percentage mortality was calculated based on the initial number of larvae within each plate.

Statistics

All percentage larval settlement and mortality data were analysed using the non-parametric Kruskal–Wallis H-test and followed by Dunn’s multiple comparison tests. The level of significance chosen was 0.05 for all statistical tests. Data were analysed using the Minitab version 16 statistical software package.

Results

L-phenylalanine and L-tyrosine induced larval settlement with similar dose-dependent response trends (Figure 1). For each amino acid, significant differences (Kruskal–Wallis) were detected among concentrations (Table II). Compared with controls, L-phenylalanine was an effective inducer at 10^{-7} , 10^{-6} and 10^{-5} mol L⁻¹ (Dunn’s tests: $P < 0.05$). Treatment with L-tyrosine gave comparable results, but also induced settlement at 10^{-8} mol L⁻¹ (Dunn’s tests: $P < 0.05$). However, inductive effects of each compound at concentrations below 10^{-5} mol L⁻¹ were minimal. Toxicity responses of larvae to L-phenylalanine and L-tyrosine revealed that neither amino acid caused significant mortality compared with controls (<20%).

Induction of settlement also was achieved with L-DOPA, dopamine and epinephrine treatments (Figure 2) with significant differences (Kruskal–Wallis) detected among concentrations (Table II). Although the range of treatment concentrations varied among the compounds tested, exposure of

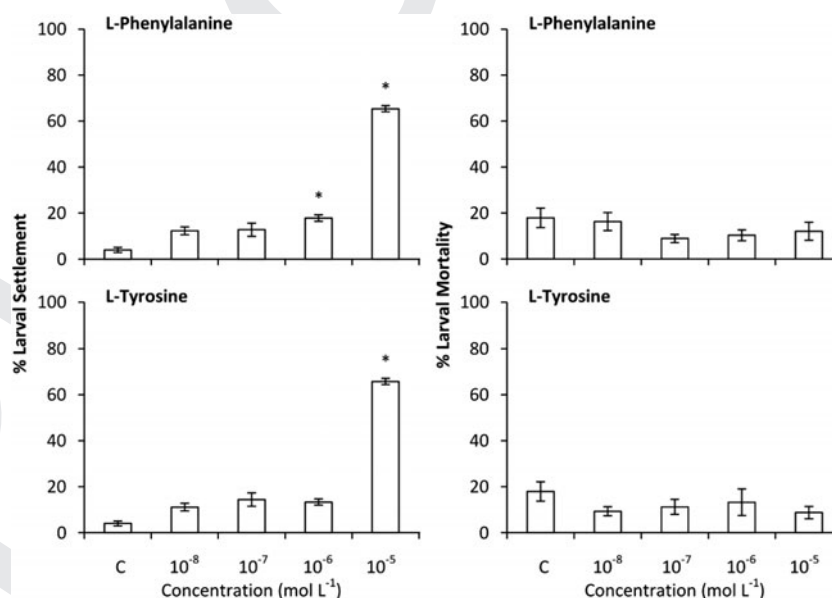


Figure 1. Percentage (\pm SE) settlement (graphs on left) and mortality (graphs on right) of mussel larvae exposed to L-phenylalanine and L-tyrosine at different concentrations. Control assays are denoted ‘C’ on the x-axes and asterisks (*) represent significant differences against controls resulting from Dunn’s multiple comparisons.

larvae to L-DOPA, dopamine and epinephrine resulted in maximum induction (50, 60 and 51% respectively) at 10^{-5} mol L⁻¹. In each case, higher concentrations resulted in a decrease in inductive effect with complete inhibition observed at 10^{-3} mol L⁻¹ for L-DOPA and epinephrine. At 10^{-5} mol L⁻¹, mortality responses to all three compounds generally were low (~20%), but were high ($\geq 60\%$) at 10^{-4} mol L⁻¹ (Figure 2).

Table II. Kruskal-Wallis statistics of larval settlement and mortality after exposure to different concentrations of catecholamines and their precursor amino acids.

Compound	Settlement			Mortality		
	df	H	P-value	df	H	P-value
L-phenylalanine	4	36.32	<0.001	4	4.98	0.290
L-tyrosine	4	35.31	<0.001	4	3.96	0.411
L-DOPA	3	32.56	<0.001	3	31.80	<0.001
Dopamine	3	20.06	<0.001	3	25.47	<0.001
Epinephrine	3	33.54	<0.001	3	32.16	<0.001

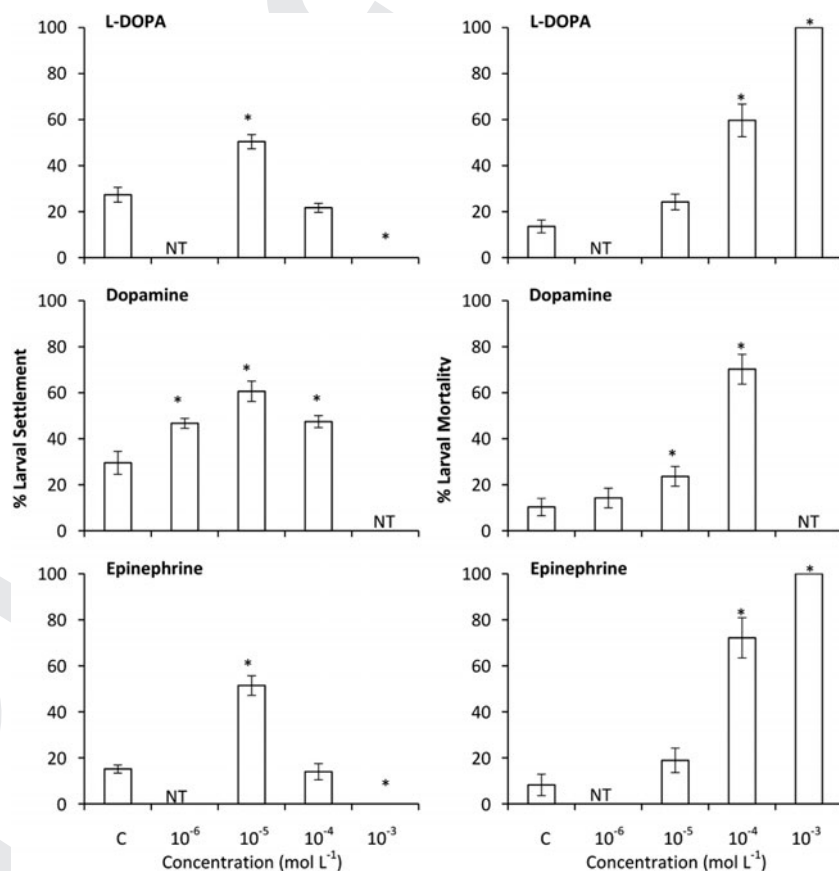


Figure 2. Percentage (\pm SE) settlement (graphs on left) and mortality (graphs on right) of mussel larvae exposed to different concentrations of L-DOPA, dopamine and epinephrine treatments. Concentrations that were not tested for some compounds are denoted with the abbreviation NT. Control assays are denoted 'C' on the x-axes and asterisks (*) represent significant differences against within-trial controls resulting from Dunn's multiple comparisons.

While the mechanism of L-phenylalanine and L-tyrosine induction is currently unknown, we tentatively hypothesize that their ability to induce settlement is *via* conversion to their neuroactive catecholamine derivatives. In support, it is well-established that treatment of marine molluscs with dopamine precursors enhances levels of endogenous catecholamines. For example, in the mussel *Mytilus edulis* (Linnaeus, 1758) and the clam *Mercenaria mercenaria* (Linnaeus, 1758), dopamine biosynthesis in pedal ganglia is stimulated by exogenous incubation with L-tyrosine (Boadle-Biber & Roth 1972; Zhu et al. 2005a). Elevated dopamine and norepinephrine levels, and an increase in the numbers of catecholamine-containing cells, have also been observed in the nudibranch *Phestilla lugubris* (Bergh, 1870) following treatment of whole organisms with the L-tyrosine–dopamine intermediate L-DOPA (Pires et al. 2000). In addition, positive immunolabelling of tyrosine hydroxylase-containing cells (the enzyme that catalyses the synthesis of L-DOPA from L-tyrosine) within the velar tissue of *Crepidula formicata* (Linnaeus, 1758) indicates the presence of a catecholamine-based settlement regulatory mechanism via innervations of propulsive cilia (Penniman et al. 2013). However, since L-phenylalanine and L-tyrosine are involved in other metabolic pathways, such as synthesis of thyroxine (a thyroid hormone implicated in regulation of echinoderm development) (Saito et al. 2012), tyramine, a biogenic amine implicated in the regulation of bryozoan larval settlement (Shimizu et al. 2000), an octopamine, norepinephrine analogue with dopaminergic and adrenergic activity (Dahlström & Elwig 2006) and morphine (Zhu 2005b), we recognize that alternative mechanisms, not yet identified, may be involved in their settlement-inducing effects. Thus, by employing a suite of traditional and contemporary methods (e.g. immunohistochemistry, confocal microscopy, metabolomics and proteomics), we are currently working on testing our hypothesis to identify the precise mode/s of action for these proteinogenic amino acids.

It has previously been demonstrated that endogenous dopamine, norepinephrine and epinephrine, or proteins involved in their synthesis, are present in appreciable quantities and exhibit unique profiles during early development of marine invertebrates (Anitole-Misleh & Brown 2004; Croll 2006; Huan et al. 2012). Levels of endogenous dopamine peak during settlement and metamorphosis in larvae of the scallop *Pecten maximus* (Linnaeus, 1758) (Cann-Moisan et al. 2002), and a steady increase in norepinephrine has been detected leading up to metamorphosis in the oyster *Crassostrea gigas* (Thunberg, 1793) (Coon & Bonar 1986). Also during this

phase of larval development, increases in numbers of catecholamine-containing cells and changes to their distributions within tissues occur in the scallop *Placopecten magellanicus* (Gmelin, 1791) and the mussel *M. edulis* (Croll et al. 1997). While such classical neurotransmitters are known to have regulatory roles through animal ontogenesis prior to development of the nervous system (Buznikov et al. 1996), these correlative evidences between neurotransmitter expression and post-ganglionic development suggest that the molecular mechanisms of settlement and metamorphosis are under dopaminergic and adrenergic neuronal control. Stimulation of these processes by manipulation of endogenous transmitter levels, or activation of target receptors, provides further evidence of catecholamine involvement.

An early model for catecholamine-induced settlement and metamorphosis in oysters has been proposed to involve regulation by dopaminergic behavioural and adrenergic morphogenetic pathways, respectively (Coon et al. 1985; Coon & Bonar 1986; Bonar et al. 1990). In an effort to identify the specific receptor/s responsible for catecholamine-induced settlement and metamorphosis in bivalves, results of pharmacological and immunohistochemical investigations provide evidence to suggest the involvement of β -adrenergic-type receptors in the central nervous systems of the clam *Meretrix meretrix* (Linnaeus, 1758) (Wang et al. 2006) and α -adrenergic-type receptors in the mussel *Mytilus coruscus* Gould, 1861 (B. Yang et al. 2014). While the role, and even presence, of epinephrine in the neural tissues of marine invertebrates has been debated (Stefano & Kream 2010), analysis of larval gene expression (DDRT-PCR) in the clam *Ruditapes philippinarum* (Adams & Reeve, 1850) revealed high levels of differentially expressed transcripts compared with control organisms after induction of settlement/metamorphosis with epinephrine (Sumin et al. 2006). In addition, Yang et al. (2012) recently characterized an adrenergic-like receptor gene (ARcga) in larvae of the oyster *Crassostrea angulata* (Lamarck, 1819) and performed a temporal analysis of its expression during larval development through real-time qPCR and whole mount *in situ* hybridization. A clear peak in ARcga expression was observed during settlement and metamorphic competency and also after bath application of whole larvae in epinephrine (Yang et al. 2012). In a subsequent investigation to identify downstream components of the epinephrine-induced pathway, Yang et al. (2014) also provided evidence for the involvement of Receptor for Activated C Kinase 1 (RACK1) during larval metamorphosis of *C. angulata* and demonstrated that its gene and protein

expressions were up-regulated by epinephrine treatment. RACK1 is known to interact with protein kinase C (PKC) and cyclic adenosine monophosphate (cAMP), both of which have been implicated as endogenous regulators of marine invertebrate larval settlement or metamorphosis (Li et al. 2008). It appears that interpretations of data generated from pharmacological-based approaches to investigate regulatory biochemical pathways and neuronal control of larval settlement are increasingly being supported by the results from molecular studies. Thus, mounting evidence indicates that at least for some bivalve species, including *P. canaliculus*, adrenergic receptors have an important role to play in the signal transduction mechanism of settlement and/or metamorphosis.

Furthermore, biogenic amines naturally present in seawater may deliver an exogenous mechanism which target endogenous or epithelial-bound larval receptors. For example, some marine biofilms release settlement-promoting compounds that are structurally related to L-DOPA (Raillkin 2004). The bloom-forming macroalga *Ulvaria obscura* (Kützing) ex P. Gayral ex C. Blinding is also known to release large amounts of dopamine into surrounding seawater (up to 550 μM) upon rehydration following emersion during low tide (Van Alstyne et al. 2011, 2013). At these environmentally relevant concentrations, there is evidence to suggest that the dopamine produced can affect cohabitating algal and invertebrate communities in the intertidal and shallow subtidal zones (Van Alstyne et al. 2014). While *U. obscura* is not found in New Zealand, it is possible that catecholamines released from algae or marine biofilms may provide an environmental source.

We previously investigated the effects of epinephrine and/or precursor metabolites in two other marine mussels, *Aulacomya maoriana* (Iredale, 1915) (Alfaro et al. 2011) and *Mytilus galloprovincialis* Lamark, 1819 (Sánchez-Lazo et al. 2012), which revealed similar settlement responses to those found for *P. canaliculus* in the present study. These similarities are suggestive of a common biochemical mechanism of larval settlement among mussel species, but more comprehensive comparative studies will be needed to identify patterns among other molluscan clades.

The identification of endogenous regulatory mechanisms of larval development rates, settlement behaviour and morphogenetic processes has wide ecological and economic implications. The distributions and survival of sessile marine invertebrates depend largely on their ability to successfully select suitable habitats and transition to their juvenile forms. Exogenous stimuli (physical, chemical or biological) that have the ability to affect these

mechanisms could cause dramatic changes in population dynamics and species compositions. For example, a wide range of anthropogenic pollutants with pharmacological activity are increasingly being detected in a variety of marine habitats, from populated urban areas to remote and exposed coastlines. Caffeine provides an interesting case study due to its familiarity, high global usage and widespread abundance in environmental waters. As a central nervous system stimulant, caffeine is known to cause neuronal release of acetylcholine and dopamine, increase norepinephrine synthesis and enhance dopaminergic neurotransmission (Acquas et al. 2002; Ferré 2010). Having been detected in marine environments at levels exceeding those which are chronically toxic for shellfish (Siegener & Chen 2002; Aguirre-Marínez et al. 2013; Beretta et al. 2014), potential effects on benthic community structure via regulation of larval behaviour and development rates seems plausible. Characterization of endogenous developmental mechanisms provides a predictive ability to hypothesize and test possible latent effects of particular anthropogenic pollutants based on their known pharmacological activities. On a more positive note, the identification of receptors involved in settlement and metamorphosis for species with commercial value offers potential means to improve production by enhancing settlement rates and synchronizing metamorphosis during hatchery culture. As one of the key bottlenecks in the growing mollusc aquaculture industry, the ability to stimulate these processes would be highly advantageous.

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