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TRPA1 expression provides new insights into thermal perception by the sea urchin *Strongylocentrotus intermedius*

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Original Article

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Abstract

Thermal perception is crucial for the fitness of marine invertebrates in intertidal and shallow waters. TRPA1 is a non-selective cation channel that belongs to the TRP family with pivotal roles in initiating signal transduction of thermal perception. We investigated expression patterns of *SiTRPA1* in different tissues (tube feet, coelomocytes, gonads and gut) of the sea urchin *Strongylocentrotus intermedius*. *SiTRPA1* expression patterns under acute and long-term temperature stimuli were investigated in tube feet of sea urchins. In the present study, the highest expression of *SiTRPA1* was detected in tube feet of *S. intermedius*. The *SiTRPA1* expression level in tube feet were significantly 235.7-fold, 450.0-fold and 3299.7-fold higher than those in the coelomocytes, gonads and gut ($df = 3$, $F = 47.382$, $P < 0.001$). Expression levels of *SiTRPA1* in the other tissues (coelomocytes, gonads and gut) were not significantly different ($df = 3$, $F = 47.382$, $P = 0.972$). There was no significant difference of *SiTRPA1* expression among all groups in the acute temperature increase experiment ($df = 4$, $F = 0.25$, $P = 0.902$). In the acute temperature decrease experiment, the expression of *SiTRPA1* showed no significant difference among all groups ($df = 4$, $F = 1.802$, $P = 0.205$). With long-term exposure (6 weeks) to different temperatures, *SiTRPA1* expression in the low temperature group (10°C) was significantly higher than those in the high temperature (20°C) and the control groups (15°C) ($df = 2$, $F = 9.57$, $P = 0.014$). There was no significant difference of *SiTRPA1* expression between the high temperature (20°C) and the control temperature (15°C) groups ($df = 2$, $F = 9.57$, $P = 0.808$). These results indicate that *SiTRPA1* expression significantly responds to long-term low temperature but not to acute temperature decrease. The present study provides new insights on the distribution and temporal expression of *TRPA1* in marine invertebrates after acute and long-term temperature stimuli.

Introduction

Marine invertebrates are exposed to both acute and long-term thermal stress in intertidal and shallow waters during the juvenile and adult periods in their life histories (Pinsky *et al.*, 2019). Migration and acclimation are the main strategies for these thermally stressed animals to avoid decrease in function or mortality (Murren *et al.*, 2015; Stillman & Armstrong, 2015; Veilleux *et al.*, 2015). Notably, both strategies are based on thermal perception. Thus, it is essential to explore the relationship between temperature stimuli and thermosensory systems under short- and long-term temperature stress in ecologically important marine invertebrates in intertidal and shallow waters.

Transient receptor potential (TRP) ion channels are key molecules in thermosensory systems and consequently important for the fitness of various organisms (Patapoutian *et al.*, 2003; Tominaga & Caterina, 2004). The temperature-responsive TRPs are designated thermo-TRPs. They consist of TRPV, TRPM and TRPA subfamilies (Patapoutian *et al.*, 2003; Jordt *et al.*, 2004). TRPA1 is a non-selective cation channel that belongs to a TRPA subfamily with pivotal roles in initiating signal transduction of thermal perception (Roessingh & Stanewsky, 2017). Activated TRPA1 probably leads to Ca^{2+} signalling and neuronal excitability after cold detection (Jordt *et al.*, 2004; Chen, 2015). *TRPA1* highly expresses in cold-sensitive neurons, and was proposed to function as a sensor of noxious cold (painful sensation caused by cold) in mice and humans (Story *et al.*, 2003; Bandell *et al.*, 2004). In addition, *TRPA1* is highly expressed in thermal sensory neurons of other vertebrates (Yun *et al.*, 2010; Yonemitsu *et al.*, 2013) and invertebrates (Lee, 2013; Luo *et al.*, 2016).

Despite the importance of *TRPA1* in the thermal detection of terrestrial animals, it has been rarely proposed as a thermal detector in marine invertebrates. Saito *et al.* (2017) found that *TRPA1* highly expressed in the neural ciliary band, as a thermal sensor, of the planktonic larvae of the starfish *Patiria pectinifera*. Acute and long-term thermal stimuli, however, were not involved in this study, leaving the role of thermal detection by *TRPA1* uninvestigated. Furthermore, to our knowledge, *TRPA1* has not been investigated in any intertidal or shallow water marine invertebrate.

Sea urchins are ecologically important in structuring marine benthic communities, both as grazers and prey (Pearse, 2006). Studies have reported that temperature changes significantly impact the fitness related traits of sea urchins (Wolfe *et al.*, 2013; Uthicke *et al.*, 2014; Bodmer *et al.*, 2017). Further, these impacts on sea urchin fitness can extend to the whole marine benthic communities, highlighting their research value for marine ecology. The sea urchin *Strongylocentrotus intermedius*, as a typical cold-water invertebrate, is distributed in intertidal and shallow waters of Hokkaido of Japan, Korea, China and Far East Russia (Agatsuma, 2013). Considering its ecological importance and exposure to thermal stress, *S. intermedius* is an ideal research model to investigate the *TRPA1* expression in response to temperature changes. Tube feet have strong sensory functions and have a complicated nerve plexus, responding to a variety of environmental stimuli (Sharp & Gray, 1962; Florey & Cahill, 1977; Ullrich-Lüter *et al.*, 2011).

The main aim of the present study is to investigate the distribution and temporal expression of *TRPA1* of *S. intermedius* after acute and long-term temperature stimuli in order to provide new insights into thermal detection of marine invertebrates. We asked (1) whether *SiTRPA1* expressed significantly more in tube feet than in other analysed tissues; (2) whether *SiTRPA1* expression significantly responds to high and/or low temperature; and (3) whether *SiTRPA1* expression significantly responds to acute temperature changes and/or long-term temperature treatments.

Materials and methods

Sea urchins

Strongylocentrotus intermedius were obtained from a local aquaculture farm and taken to the Key Laboratory of Mariculture & Stock Enhancement in the North China's Sea, Ministry of Agriculture and Rural Affairs at Dalian Ocean University. Nine sea urchins were used for analysis of *SiTRPA1* in different tissues. Analysis of acute and long-term temperature stimuli were performed with 108 individuals in total (body weight = 9.99 ± 0.18 g). In order to ensure that the sea urchins were randomly selected for each experiment, individual sea urchins were placed into a labelled plastic container. A random number generator was used to obtain the sea urchins corresponding to the random number. The method was repeated until 108 individuals were randomly selected. The sea urchins were maintained at 16–17°C in the laboratory in tanks with inflowing ambient sand filtered sea water. The salinity was 28–30‰, the pH was 8.03–8.26 and dissolved oxygen was 6.2–7.0 mg l^{-1}. The photoperiod was 12 h light: 12 h dark. Aeration was provided continuously. Sea urchins were not fed during the experiments

Sample collection for transcriptional analysis of SiTRPA1

In order to investigate the expression pattern of *SiTRPA1* transcripts in different tissues of *S. intermedius*, tube feet from the aboral side (Lesser *et al.*, 2011), coelomocytes, gonads and gut were carefully collected from nine *S. intermedius* (body weight = 23.41 ± 0.63 g). Tissues of 3 individuals were mixed as one sample (N = 3) (Li *et al.*, 2017). Coelomic fluid samples were centrifuged immediately at 3000 × rpm for 2 min (4°C). The tissue samples and the coelomocytes were immediately snap-frozen in liquid nitrogen, and stored at −80°C until used for RNA extraction.

Acute temperature rise and decrease treatments

Generally, for *S. intermedius* the upper lethal temperature and optimal temperatures are ~23 and ~15°C, respectively (Chang *et al.*, 1999). Thus, two target temperatures were set as 12 and 22°C. Water temperatures in acute temperature increase and decrease treatments were increased from 17 to 22°C and decreased from 17 to 12°C within 2 h at a rate of 2.5°C h^{-1}. Four independent samples were taken at 1, 3, 6 and 12 h after achieving the target temperatures (12 and 22°C). According to the experimental design, 72 sea urchins (body weight = 10.09 ± 0.22 g) were randomly selected and equally distributed into eight temperature-controlled tanks (750 × 430 × 430 mm; Huixin Co., Dalian, China) before the temperature change. Each temperature-controlled tank contained nine containers made of a plastic screen (200 × 200 × 400 mm, mesh size: 8 mm), each with an individual sea urchin. In addition, nine sea urchins (body weight = 9.45 ± 0.51 g) were individually placed into nine containers made of a plastic screen (200 × 200 × 400 mm, mesh size: 8 mm) in a temperature-controlled tank (17°C) as the control group. Tube feet were dissected from the aboral side (Lesser *et al.*, 2011) at 1, 3, 6 and 12 h after the target had been reached (three individuals mixed as one sample, N = 3) (Li *et al.*, 2017). All samples were immediately frozen by liquid nitrogen and stored at −80°C for subsequent RNA extraction.

Long-term high and low temperature treatments

Three treatments were set in the long-term temperature stimuli experiment as: high temperature (20°C), control temperature (15°C) and low temperature (10°C). Twenty-seven sea urchins (body weight = 9.92 ± 0.34 g) were randomly selected and equally distributed into three treatments. Nine individuals of each treatment were placed into individual containers made of a plastic screen (200 × 200 × 400 mm, mesh size: 8 mm) inside three temperature-controlled tanks (750 × 430 × 430 mm; Huixin Co., Dalian, China). Prior to the experiments, the sea urchins were maintained under 17°C for 24 h. After this period, seawater temperature was increased or decreased at a rate of 0.5°C day^{-1} to the target temperatures (20, 15 and 10°C). After the target temperatures were reached, the sea urchins were cultured for 6 weeks. At the end of the experiment, tube feet of each individual were collected from the aboral side (Lesser *et al.*, 2011) (three individuals mixed as one sample, N = 3) (Li *et al.*, 2017). All samples were immediately frozen in liquid nitrogen and stored at −80°C before RNA extraction.

Total RNA extraction and cDNA synthesis

Total RNA was extracted using the RNAprep Pure Tissue Kit (Tiangen, China) according to the manufacturer's instructions. The integrity of RNA was assessed by agarose gel electrophoresis. The concentration and quality of RNA were determined using a NanoPhotometer (Implen GmbH, Germany). The cDNA was synthesized using PrimeScript™ RT reagent Kit (TaKaRa, Japan). The PCR reaction system was carried out in a total volume of 20 µl composed of 1000 ng total RNA, 4 µl 5 × PrimeScript™ buffer, 50 pmol Oligo dT Primer, 100 pmol Random 6 mers and 1 µl PrimeScript RT Enzyme Mix I. The thermal condition of reaction was incubated at 37°C for 15 min, and then at 85°C for 5 s to deactivate the enzyme. All cDNA products were stored at −20°C for subsequent SYBR Green fluorescent quantitative real-time PCR.

Transcriptional analysis of SiTRPA1 by quantitative real-time PCR

The *SiTRPA1* expression levels were analysed with quantitative real-time PCR (qRT-PCR), which was conducted with the Applied Biosystem 7500 Real-time System (Applied Biosystems, Foster City, CA, USA). Gene-specific primers were designed for the *SiTRPA1* fragments using Primer Premier 5.0 software. The

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Table 1. PCR primers used in this study

Primers	Sequnences (5′–3′)	Application
SiTRPA1-F	GCCACCGCAGTCGTGTGTG	qPCR
SiTRPA1-R	TGGGCGTGGTCCGATAGTTAGTCTC	qPCR
18S rRNA-F	GTTCGAAGGCGATCAGATAC	Reference gene
18S rRNA-R	CTGTCAATCCTCACTGTGTC	Reference gene

18 S gene was used as the reference gene in this study (Table 1) (Zhou *et al.*, 2008; Han *et al.*, 2019). According to the manufacturer's instructions, real-time PCR was carried out in a total volume of 20 µl containing 1 µl of 1:5 diluted original cDNA, 7 µl ddH_2O, 10 µl of 2 × SYBR Green Master mix (SYBR PrimeScript × RT-PCR kit II, TaKaRa, Japan), 0.4 µl of ROX Reference Dye II, 0.4 µM of each primer (Table 1). The qRT-PCR cycling protocol was 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, and 60°C for 32 s. At the end of the PCR reactions, melting curve analysis of the amplification products confirmed that a single PCR product was present in these reactions (Han *et al.*, 2019). The comparative Ct method ($2^{-\triangle\triangle CT}$ method) was used to calculate the relative expression levels of the target genes (Livak & Schmittgen, 2001).

Statistical analysis

Data were tested for normal distribution and homogeneity of variance before statistical analysis. Gene expression was analysed using one-way ANOVA. When a significant effect was found, Duncan multiple comparisons were conducted to compare significant differences among treatments. All data are expressed as mean values ± standard error (mean ± SE). All statistical analysis was performed using SPSS 16.0 statistical software. The level of significance was considered as $P < 0.05$.

Results

Tissues distribution of SiTRPA1 *mRNA*

The expression of *SiTRPA1* in tube feet was 235.7-fold, 450.0-fold and 3299.7-fold significantly higher than those in the coelomocytes, gonads and gut (df = 3, $F = 47.382$, $P < 0.001$, Figure 1). But the *SiTRPA1* expression were not significantly different among coelomocytes, gonads and gut (df = 3, $F = 47.382$, $P = 0.972$, Figure 1).

Expression pattern of SiTRPA1 *after acute temperature increase stimuli*

No significant difference of *SiTRPA1* expression was found among all groups in the acute temperature increase experiment (df = 4, $F = 0.25$, $P = 0.902$, Figure 2).

Expression pattern of SiTRPA1 *after acute temperature decrease stimuli*

The expression of *SiTRPA1* showed no significant difference among all groups in the acute temperature decrease experiment (df = 4, $F = 1.802$, $P = 0.205$) (Figure 3).

Long-term effects of high and low temperatures on SiTRPA1 *expression pattern*

In the long-term temperature experiment, *SiTRPA1* expression in the low temperature group (10°C) was significantly higher than those in the high temperature (20°C) and control (15°C) groups (df = 2, $F = 9.57$, $P = 0.014$, Figure 4). However, there was no significant difference in the expression of *SiTRPA1* between the high temperature (20°C) and control group (15°C) (df = 2, $F = 9.57$, $P = 0.808$, Figure 4).

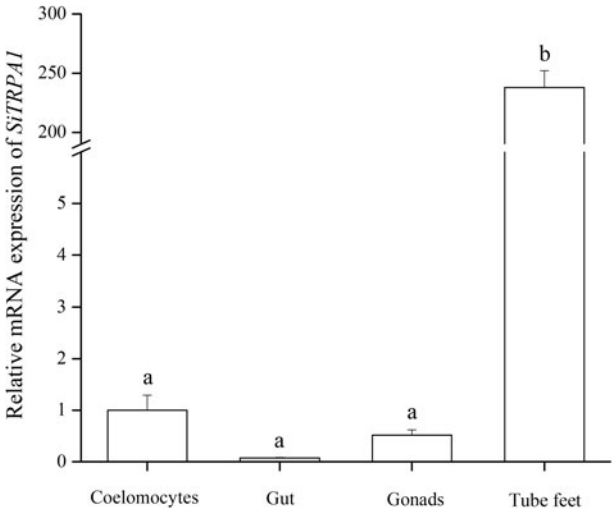

Fig. 1. Gene expression of *SiTRPA1* in different tissues (N = 3, mean ± SE).
Notes: Different letters above the bars indicate significant differences in different tissues ($P < 0.05$).

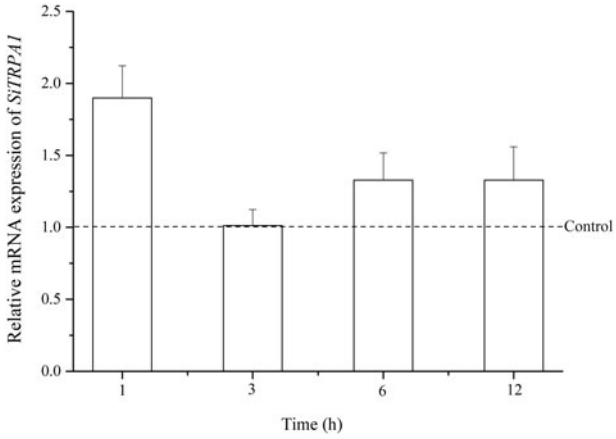

Fig. 2. Gene expression of *SiTRPA1* of tube feet (from the aboral side) in different time points after acute temperature rise treatment (N = 3, mean ± SE).
Notes: The relative mRNA expression of *SiTRPA1* of control group was not under temperature stress.

Discussion

During the process of thermal perception, expression of *TRPA1* is significantly highest in the organs that contain the most abundant sensory neurons (Lee, 2013; Luo *et al.*, 2016) in a number of animals, including the antennae of the insect *Apolygus lucorum* (Fu *et al.*, 2015) and the ciliary band of the starfish *P. pectinifera* larvae (Saito *et al.*, 2017). In the present study, the highest expression of *SiTRPA1* was detected in the tube feet among the experimented tissues of *S. intermedius*. Similar to antennae of *A. lucorum* and ciliary band of starfish *P. pectinifera*, tube feet as a functional tissue are essential for the sea urchin's locomotion and feeding behaviour (Ullrich-Lüter *et al.*, 2011). This indicates that tube feet play an essential role in thermal perception of *S. intermedius*, although radial nerves and nerve ring tissues

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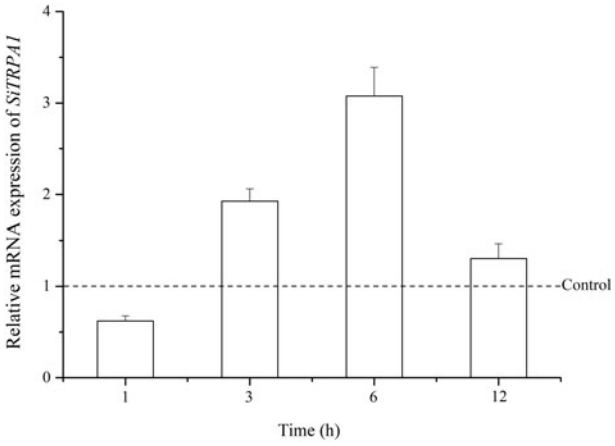

Fig. 3. Gene expression of *SiTRPA1* of tube feet (from the aboral side) in different time point of acute temperature decrease treatment (N = 3, mean ± SE).
Notes: The relative mRNA expression of *SiTRPA1* of control group was not under temperature stress.

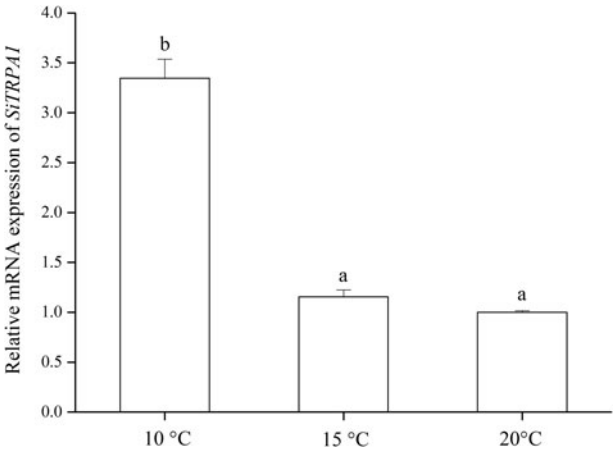

Fig. 4. Gene expression of *SiTRPA1* of tube feet (from the aboral side) in different long-term temperature treatments (N = 3, mean ± SE).
Notes: Different letters above the bars indicate significant differences ($P < 0.05$).

were not involved in the present study. However, those tissues lack well-developed musculature and sensory neurons would be insensitive to external temperature changes (Sharp & Gray, 1962; Florey & Cahill, 1977). This would account for the very low level of *SiTRPA1* expression in coelomocytes, gonads and gut compared with tube feet.

Generally, TRPA1 is well documented as a cold receptor rather than a heat receptor (Bandell *et al.*, 2004; Jordt *et al.*, 2004; Caspani & Heppenstall, 2009). Consistently, *SiTRPA1* expression was insensitive to high temperature stimuli (acute temperature and long-term increase temperature) in *S. intermedius*. Chen *et al.* (2013) reported that the Glycine (G) residue is critical for cold-activation of TRPA1 (e.g. rat), whereas an equivalent Valine (V) residue is preserved in heat-insensitive (e.g. human), as well as in heat-activated TRPA1 channel (e.g. rattlesnake). This suggests that the structure of the TRPA1 channel itself exhibits divergence between different species. *SiTRPA1* expression was insensitive to high temperature stimuli in *S. intermedius*. It is essential to sequence transcripts of *SiTRPA1* to further test this phenomenon in the future. Another possibility is that other members of the thermo-TRPs rather than *SiTRPA1* may be involved in the heat perception. Caterina *et al.* (1997) reported that heat-activated nociceptors are characterized by the expression of the heat- and capsaicin-activated channel TRPV1. In addition, *TRPV1*$^{-/-}$ mice significantly reduced or even lost their perception of heat (Caterina, 2007). *TRPV1* blockers could increase the body temperature in animals and humans, suggesting that *TRPV1* is a necessary thermosensor involved in body temperature regulation (Caterina, 2007; Cheng *et al.*, 2007; Gavva *et al.*, 2008). Further, Chen (2015) inferred that the gain or loss of heat sensitivity of *TRPA1* may coincide with the emergence of other thermal sensitive TRP ion channels such as TRPV1. Consistent with Chen's (2015) finding, Vandewauw *et al.* (2018) proved that *TRPA1*, *TRPV1* and *TRPM3* of mice had critical but redundant roles in heat perception, in which the functionality of at least one of the three channels was necessary and sufficient to sustain robust heat responses in sensory neurons. The presence of three redundant molecules in the nociceptors with overlapping expressions of heat-sensing mechanism neurons represents a powerful fail-safe mechanism (Vandewauw *et al.*, 2018). Based on these findings, we infer that the similar powerful fail-safe mechanism may exist in *S. intermedius*. This information provides new insights into our understanding of why *SiTRPA1* expression was not affected by high temperature stimuli (acute temperature increase and long-term elevated temperature). Further investigations are essential to test it.

Although *SiTRPA1* expression was influenced by long-term cold stimulation, it did not respond to acute cold stimulation. Story *et al.* (2003) found that *TRPA1* and *TRPM8* act as primary cold sensors in two distinct subsets of cold-sensitive trigeminal ganglion neurons of mice. Consistently, Chen (2015) inferred that the establishment of *TRPM8* may allow *TRPA1* to diverge from acute cold sensing and concentrate on another core function of sensing of noxious, reactive electrophiles. Further, Vandewauw *et al.* (2018) reported that the *TRPM8* was preserved in the trigeminal ganglia of *TRPV1*$^{-/-}$*TRPM3*$^{-/-}$*TRPA1*$^{-/-}$ triple knockout mice, thus accepting acute cold stimuli. All the findings above support our finding that *SiTRPA1* expression is insensitive to acute cold stimulation and may be related to the presence of *SiTRPM8*, although the mechanism remains unknown.

In conclusion, the present study is the first investigation on the distribution and expression of *SiTRPA1* after acute and long-term temperature stimuli in adult marine invertebrates. Unsurprisingly, the highest expression of *SiTRPA1* was detected in tube feet among the tissues analysed. *SiTRPA1* expression was insensitive to high temperature stimuli (acute temperature increase and long-term high temperature exposure). Further, *SiTRPA1* expression significantly responded to long-term low temperature treatment, although it did not significantly respond to acute temperature decrease stimulation. This present study provides new insights into the thermal detection of sea urchins.

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References

Agatsuma Y (2013) *Strongylocentrotus intermedius*. In Lawrence JM (ed.), *Edible Sea Urchins: Biology and Ecology*, 3rd Edn. San Diego, CA: Academic Press, pp. 437–447.

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ and Patapoutian A (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron* **41**, 849–857.

Bodmer MDV, Wheeler PM, Hendrix AM, Cesarano DN, East AS and Exton DA (2017) Interacting effects of temperature, habitat and phenotype

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on predator avoidance behaviour in *Diadema antillarum*: implications for restorative conservation. *Marine Ecology: Progress Series* **566**, 105–115.

Caspani O and Heppenstall PA (2009) TRPA1 and cold transduction: an unresolved issue? *Journal of General Physiology* **133**, 245–249.

Caterina MJ (2007) Transient receptor potential ion channels as participants in thermosensation and thermoregulation. *American Journal of Physiology* **292**, R64–R76.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD and Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* **389**, 816–824.

Chang YQ, Wang ZC and Wang GJ (1999) Effect of temperature and algae on feeding and growth in sea urchin, *Strongylocentrotus intermedius*. *Journal of Fisheries of China* **23**, 69–76. (In Chinese with English Abstract.)

Chen J (2015) The evolutionary divergence of TRPA1 channel: heat-sensitive, cold-sensitive and temperature-insensitive. *Temperature* **2**, 158–159.

Chen J, Kang D, Xu J, Lake M, Hogan JO, Sun C, Walter K, Yao B and Kim D (2013) Species differences and molecular determinant of TRPA1 cold sensitivity. *Nature Communications* **4**, 2501.

Cheng W, Yang F, Takanishi C and Zheng J (2007) Thermosensitive TRPV channel subunits coassemble into heteromeric channels with intermediate conductance and gating properties. *Journal of General Physiology* **129**, 191–207.

Florey E and Cahill MA (1977) Ultrastructure of sea urchin tube feet. *Cell & Tissue Research* **177**, 195–214.

Fu T, Yang T, Liu Y and Wang GR (2015) Identification and functional characterization of TRPA1 in *Apolygus lucorum* (Meyer-Dür). *Scientia Agricultura Sinica* **25**, 370–384. (In Chinese with English Abstract.)

Gavva NR, Treanor J, Garami A, Fang L, Surapaneni S, Akrami A, Alvarez F, Bak A, Darling M, Gore A, Jang G, Kesslak J, Ni L, Norman HM, Palluconi GJ, Rose M, Salfi M, Tan E, Romanovsky A and Davar G (2008) Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain* **136**, 202–210.

Han LS, Ding J, Wang H, Zuo RT, Quan ZJ, Fan ZH, Liu QD and Chang YQ (2019) Molecular characterization and expression of SiFad1 in the sea urchin (*Strongylocentrotus intermedius*). *Gene* **705**, 133–141.

Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Högestätt ED, Meng ID and Julius D (2004) Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* **427**, 260–265.

Lee Y (2013) Contribution of *Drosophila* TRPA1-expressing neurons to circadian locomotor activity patterns. *PLoS ONE* **8**, e85189.

Lesser MP, Carleton KL, Böttger SA, Barry TM and Walker CW (2011) Sea urchin tube feet are photosensory organs that express a rhabdomeric-like opsin and PAX6. *Proceedings of the Royal Society B: Biological Sciences* **278**, 3371–3379.

Li KQ, Liu L, Shang SN, Wang Y, Zhan YY, Song J, Zhang XX and Chang YQ (2017) cDNA cloning, expression and immune function analysis of a novel Rac1 gene (*AjRac*1) in the sea cucumber *Apostichopus japonicas*. *Fish & Shellfish Immunology* **69**, 218e226.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.

Luo J, Shen WL and Montell C (2016) TRPA1 mediates sensing the rate of temperature change in *Drosophila* larvae. *Nature Neuroscience* **20**, 34–41.

Murren CJ, Auld JR, Callahan H, Ghalambor CK, Handelsman CA, Heskel MA, Kingsolver JG, Maclean HJ, Masel J, Maughan H, Pfennig DW, Relyea RA, Seiter S, Snell-Rood E, Steiner UK and Schlichting CD (2015) Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity* **115**, 293–301.

Patapoutian A, Peier AM, Story GM and Viswanath V (2003) ThermoTRP channels and beyond: mechanisms of temperature sensation. *Nature Reviews Neuroscience* **4**, 529–539.

Pearse JS (2006) Ecological role of purple sea urchins. *Science* **314**, 940–941.

Pinsky ML, Eikeset AM, McCauley DJ, Payne JL and Sunday JM (2019) Greater vulnerability to warming of marine *vs* terrestrial ectotherms. *Nature* **569**, 108–111.

Roessingh S and Stanewsky R (2017) The drosophila TRPA1 channel and neuronal circuits controlling rhythmic behaviours and sleep in response to environmental temperature. *International Journal of Molecular Sciences* **18**, 2028.

Saito S, Hamanaka G, Kawai N, Furukawa R, Gojobori J, Tominaga M, Kaneko H and Satta Y (2017) Characterization of TRPA channels in the starfish *Patiria pectinifera*: involvement of thermally activated TRPA1 in thermotaxis in marine planktonic larvae. *Scientific Reports* **7**, 2173.

Sharp DT and Gray IE (1962) Studies on factors affecting the local distribution of two sea urchins, *Arbacia punctulata* and *Lytechinus variegatus*. *Ecology* **43**, 309–313.

Stillman JH and Armstrong E (2015) Genomics are transforming our understanding of responses to climate change. *Bioscience* **65**, 237–246.

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR, Earley TJ, Hergarden AC, Andersson DA and Hwang SW (2003) ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell* **112**, 819–829.

Tominaga M and Caterina MJ (2004) Thermosensation and pain. *Journal of Neurobiology* **61**, 3–12.

Ullrich-Lüter E, Dupont S, Arboleda E, Hausen H and Arnone M (2011) Unique system of photoreceptors in sea urchin tube feet. *Proceedings of the National Academy of Sciences USA* **108**, 8367–8372.

Uthicke S, Liddy M, Nguyen HD and Byrne M (2014) Interactive effects of near-future temperature increase and ocean acidification on physiology and gonad development in adult Pacific sea urchin, *Echinometra sp*. A. *Coral Reefs* **33**, 831–845.

Vandewauw I, De Clercq K, Mulier M, Held K, Pinto S, Van Ranst N, Segal A, Voet T, Vennekens R, Zimmermann K, Vriens J and Voets T (2018) A TRP channel trio mediates acute noxious heat sensing. *Nature* **555**, 662–666.

Veilleux HD, Ryu T, Donelson JM, Herwerden LV, Seridi L, Ghosheh Y, Berumen ML, Leggat W, Ravasi T and Munday PL (2015) Molecular processes of transgenerational acclimation to a warming ocean. *Nature Climate Change* **5**, 1074–1078.

Wolfe K, Dworjanyn SA and Byrne M (2013) Effects of ocean warming and acidification on survival, growth and skeletal development in the early benthic juvenile sea urchin (*Heliocidaris erythrogramma*). *Global Change Biology* **19**, 2698–2707.

Yonemitsu T, Kuroki C, Takahashi N, Mori Y, Kanmura Y, Kashiwadani H, Ootsuka Y and Kuwaki T (2013) TRPA1 detects environmental chemicals and induces avoidance behavior and arousal from sleep. *Scientific Reports* **3**, 3100.

Yun SK, Son JY, Kim TH, Sang KP, Yi D, Noguchi K, Dong KA and Yong CB (2010) Expression of transient receptor potential ankyrin 1 (TRPA1) in the rat trigeminal sensory afferents and spinal dorsal horn. *Journal of Comparative Neurology* **518**, 687–698.

Zhou ZC, Bao ZM, Dong Y, Wang LM, He CB and Liu WD (2008) *MYP* gene expressions at transcription level in different stages of gonads of sea urchin *Strongylocentrotus intermedius* and hybrids. *Hereditas* **30**, 1453–1458. (In Chinese with English Abstract.)

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