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RESEARCH ARTICLE

Iodine accumulation in sea urchin larvae is dependent on peroxide

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SUMMARY

Iodine has many important biological functions and its concentrations vary with the environment. Recent research has provided novel insights into iodine uptake mechanisms in marine bacteria and kelp through hydrogen peroxide-dependent diffusion (PDD). This mechanism is distinct from sodium-dependent mechanisms known from vertebrates. In vertebrates, iodine accumulates in the thyroid gland by the action of the apical iodide transporter (AIT) and the sodium/iodide symporter (NIS). Neither of these proteins has, thus far, been identified outside of the chordates, and PDD (as an iodine uptake mechanism) has never been studied in animals. Using ^{125}I as a marker for total iodine influx, we tested iodine uptake *via* sodium-dependent transport *versus* PDD in embryos and larvae of the sea urchin *Strongylocentrotus purpuratus*. We found that iodine uptake in *S. purpuratus* is largely independent of NIS/AIT. Instead, we found that uptake is dependent on the presence and production of hydrogen peroxide, indicating that sea urchin larvae use PDD as a mechanism for iodine acquisition. Our data, for the first time, provide conclusive evidence for this mechanism in an animal. Furthermore, our data provide preliminary evidence that sodium-dependent iodine uptake *via* active transporter proteins is a synapomorphy of vertebrates.

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INTRODUCTION

Iodine is an important trace element with functions in many biological processes. For example, iodine is essential for the synthesis of thyroid hormones (THs), a group of developmental and metabolic hormones that are produced in the vertebrate thyroid gland (Zoeller et al., 2007). Iodine also functions independently of THs, predominantly through its action as an inorganic antioxidant (Küpper et al., 2008; Aceves et al., 2005; Berking et al., 2005; Shrivastava et al., 2006). This element occurs in several oxidative states within aqueous environments. These include di-iodine (I_2; oxidation state: 0), hypoiodous acid (HIO; oxidation state: +1), iodide (I^-, oxidation state: −1) and iodate (IO_3, oxidation state: +5). The last two have been shown to be most prevalent in seawater (Wong, 1991). For simplicity, we use 'iodide' for I^- and 'iodine' for any other form of the element.

The concentration of iodine can vary drastically between freshwater and seawater ecosystems, with average values ranging from 39.4 nmol l^{-1} in surface freshwater to 453.5 nmol l^{-1} in surface seawater (Fuge and Johnson, 1986). Such variation in iodine availability is likely to affect mechanisms of iodine acquisition and its retention in aquatic organisms. The latter has been documented in teleost fish. Iodine enters teleosts through ingestion of food (e.g. Moren et al., 2008) and by diffusion across gill surfaces (Hunn and Fromm, 1966). Furthermore, data indicate that even in freshwater environments where the concentration gradient favors movement of iodine out of the organism, iodine can be actively retained within the tissue (Blanton and Specker, 2007). The same can be said for marine environments [e.g. marine copepods can contain iodine concentrations of ~94 mmol l^{-1} (Moren et al., 2006)].

Iodine transport is best characterized in the thyroid gland of vertebrates. Specifically, two members of the sodium/solute symporter family 5 (SSF5) coordinate the uptake of iodine through the basolateral and the apical plasma membrane of thyrocytes before it accumulates in the follicular lumen (Smanik et al., 1996; Rodriguez et al., 2002). These two SSF5 proteins are the sodium/iodide symporter (NIS; SLC5A5) and the apical iodide transporter (AIT; SLC5A8) (Eskandari et al., 1997; Lacroix et al., 2004). NIS and AIT share 70% sequence similarity at the amino acid level and are both inhibited by potassium perchlorate, which competitively blocks their iodide transport site (Eskandari et al., 1997; Rodriguez et al., 2002; Dohan et al., 2003). While NIS is an active transporter protein for iodine, AIT moves iodine passively along its concentration gradient (Eskandari et al., 1997; Rodriguez et al., 2002). Ecdysozoans such as the fruit fly (*Drosophila melanogaster*) do not appear to possess orthologs of NIS, based on BLAST searches and sequence analyses (Campbell et al., 2004), despite the fact that SSF5 is widespread across both prokaryotes and eukaryotes (Wright and Turk, 2004). However, NIS orthologs were reported in cephalochordates (Paris et al., 2008) and urochordates (Campbell et al., 2004), suggesting that this gene is restricted to Chordates.

Non-hormonal iodide acts as an antioxidant in Cnidarians (*Aurelia aurita*) and in *Laminaria* (Berking et al., 2005; Kupper et al., 2008), and hypothetically in mammalian physiology as well (Aceves et al., 2005; Shrivastava et al., 2006) (reviewed by Venturi and Venturi, 2009). With most reactive oxygen species (ROS), iodine is more reactive than other halogens such as bromide and chloride (Luther et al., 1995; Küpper et al., 2008). However, the reaction of iodide with hydrogen peroxide (H_2O_2) occurs slowly in the absence of an enzyme (Luther et al., 1995), and peroxidase activity accelerates this reaction allowing iodide to act as an electron donor and scavenge H_2O_2 (Luther et al., 1995; Smyth, 2003). This reaction is essential to

peroxide-dependent diffusion (PDD) of iodine first characterized in marine bacteria (Amachi et al., 2007) and kelp (Kupper et al., 1998). In both taxa, iodide from seawater is oxidized extracellularly by haloperoxidases, using H_2O_2 as a substrate. The product of this reaction has been suggested to be hypoiodous acid (HIO) (Amachi et al., 2007). Because of its reactivity it is, however, unlikely that HOI can cross the membrane and further work is required to identify the chemical form of iodine that is transported through the membrane.

Neither NIS/AIT nor PDD has been systematically investigated as an iodine uptake mechanism in any marine invertebrate species. Here, we characterized iodine acquisition in several stages of the purple sea urchin (*Strongylocentrotus purpuratus*). Previous work on sea urchin larvae has demonstrated iodine and TH metabolism within echinoids (Chino et al., 1994; Johnson and Cartwright, 1996; Saito et al., 1998; Heyland et al., 2004; Heyland and Hodin, 2004; Heyland et al., 2006). Specifically, developmental effects of THs and TH synthesis have been documented. Furthermore, the genome of the purple urchin (Sodergren et al., 2006) reveals genes orthologous to TH synthesis, metabolism and signaling genes from mammalian species (Heyland et al., 2011). Our results provide conclusive evidence for PDD in sea urchin larvae and, while sodium-dependent uptake cannot be ruled out as a mechanism in sea urchin iodine uptake, it appears to be less important.

MATERIALS AND METHODS

Animals

Adult *S. purpuratus* (Stimpson 1857) were purchased from the Cultured Abalone (Goleta, CA, USA) and were maintained in filtered synthetic seawater (Instant Ocean Aquarium Systems Inc., Mentor, OH, USA) in conditions that mimic their natural environment, i.e. at 12°C on a 8 h:16 h light:dark cycle, at the Hagen Aqualab, University of Guelph, and were fed rehydrated *Laminaria* spp. (kombu) once a week (purchased from http://www.canadiankelp.com). Experimental larvae were reared across 10, 1 male×1 female crosses from May 2011 to July 2012. Adults were spawned by agitation (Emlet, 1986). Eggs were collected by inversion into 0.2 µm-filtered Instant Ocean seawater (FSW) and sperm was collected dry. Eggs were washed three times and fertilized at 12°C and embryos were reared in cultures of ~1000 larvae in 1800 ml of FSW at 12°C. The water was changed three times a week, at which time the sea urchins were fed a diet of *Isochrysis galbana* Parke 1949 (12,000 cells ml^{-1}) and *Dunaliella teritolecta* Butcher 1959 (6000 cells ml^{-1}).

To observe potential changes in iodine influx during the metamorphic transition, larvae were reared to metamorphic competence (~8 weeks post-fertilization) and were exposed to biofilm to induce metamorphosis. Larvae were incubated in 8 ml of FSW in dishes that had been exposed for 6 weeks to FSW from the adult rearing tanks and contained a layer of biofilm. After an overnight incubation, juveniles were collected and any individuals that had not undergone metamorphosis were also collected and kept separately.

Iodine uptake experiments

Iodine influx experiments were conducted in artificial seawater (ASW: 470.57 mmol l^{-1} NaCl, 275.90 mmol l^{-1} $MgSO_4 \cdot 7H_2O$, 7.75 mmol l^{-1} $CaCl_2 \cdot H_2O$, 26.56 mmol l^{-1} $Cl_2Mg \cdot 6H_2O$, 10.05 mmol l^{-1} KCl, 2.5 mmol l^{-1} $NaHCO_3$, 0.84 mmol l^{-1} KBr, 0.01 mmol l^{-1} $SrCl \cdot 6H_2O$, 0.04 mmol l^{-1} H_3BO_3) at 35 p.p.t., pH 8 and 14°C unless otherwise specified.

Iodide in the seawater used for the incubation experiments was measured by cathodic stripping square wave voltammetry (CSSWV) and differential pulse polarography (DPP), respectively, using a Metrohm 746VA Trace Analyzer (Metrohm AG, Herisau, Switzerland) (Herring and Liss, 1974; Campos, 1997). Trace amounts of iodine from salts used in ASW added up to 100 nmol l^{-1} based on this method. Therefore, this was the concentration of iodide in ASW unless otherwise specified.

To observe iodine influx patterns during fertilization, eggs were spawned into ASW at 12°C containing 269.25 µmol l^{-1} of sodium ampicillin (Fisher, Fair Lawn, NJ, USA) and washed three times in this medium. This antibiotic was used to decrease the populations of any marine bacteria that may potentially incorporate iodine and therefore confound the results. Ampicillin was removed prior to fertilization with a further wash in ASW. Dry-collected sperm was activated in ASW and fertilization success was >90%.

Six-arm stage larvae used for iodine incorporation experiments were starved overnight in ASW to allow the larvae to clear any live algal cells from their stomach; 269.25 µmol l^{-1} of sodium ampicillin was included to limit bacterial growth. Larvae were transferred into 70 µm cell-strainers (product no. 32350, Vacutainer Labware Medical, Franklin Lakes, NJ, USA), the water was drained and strainers were moved into new, sterile ASW before treatment.

Iodine influx experiment protocol

Pharmacology experiments were conducted on fertilized eggs, six-arm stage larvae (~3 weeks post-fertilization) and metamorphically competent larvae and juveniles. The large majority of experiments were performed on six-arm stage larvae as our preliminary work determined that later stages are easier to handle than embryos in the uptake assays. In these experiments, 50 larvae were used per replicate and ~350 eggs per replicate.

Eggs and larvae were pulse centrifuged to a maximum speed of 3000 relative centrifugal force (r.c.f.; Model 5430 benchtop centrifuge, Eppendorf, Hamburg, Germany) as this was found to be effective in concentrating larvae without damaging them. The ASW supernatant was replaced with the treatment media for pre-incubation, if necessary. For each exposure, 37 kBq ml^{-1} of Na-^{125}I (Perkin Elmer, Woodbridge, ON, Canada) in ASW (^{125}ASW) (Klebanoff et al., 1979) was used and each replicate was incubated in 0.5 ml of ^{125}ASW. After ^{125}ASW incubation, eggs/larvae were pelleted by centrifugation (3000 r.c.f.) and washed with ice-cold ASW containing 1 mmol l^{-1} potassium iodide (Sigma, Oakville, ON, Canada) until the supernatant activity was at background levels (<30 c.p.m.). Samples were kept on ice during the washes. Activity per replicate (represented as c.p.m. after a 60 s measurement time) was measured using a Perkin Elmer Wizard2 Automatic Gamma counter (Model 2470, Perkin Elmer, Waltham, MA, USA).

Kinetics of iodine influx

To assess the kinetics of iodine influx, influx rate was measured as a function of time and iodine concentration. We measured ^{125}I content after 2.5, 5, 10, 20, 40, 80 and 160 min incubations in ^{125}ASW containing 2000 nmol l^{-1} potassium iodide (KI) to determine when the initial iodine influx rate occurs. To observe the effect of environmental iodine concentration on iodine uptake, we calculated the initial influx in response to KI concentrations ranging from 100 to 1800 nmol l^{-1}. The initial influx rate was determined from the initial slopes (occurring between 2.5 and 10 min) of the transport kinetic curves. The initial influx rate from the time experiment revealed an optimal influx rate at 5 min of exposure in ^{125}ASW, though a 2.5 min incubation did provide a detectable signal. To calculate the effect of temperature on *S. purpuratus* iodine uptake, larval ^{125}I influx was measured at 4, 14 and 21°C . The effect of pH on iodine influx was determined by incubating larvae for 2.5 min in ^{125}ASW in one of four pH conditions: 8, 7.5, 7 and 6.5.

To determine the effect of metamorphosis on iodine uptake, competent larvae were induced to undergo metamorphosis using naturally grown biofilm and the iodine influx rate was measured for these stages. Competent larvae and induced individuals (i.e. those exposed to biofilm) were incubated for 2 h in ASW containing 269.25 μmol l^{-1} of sodium ampicillin prior to the iodine uptake experiment. The experiment had four developmental groups with three replicates per group and 10 individuals per replicate: competent larvae (uninduced), pre-competent larvae (induced but did not metamorphose), juveniles 8 h post-induction and juveniles 10 days post-induction. Replicates were incubated for 30 min in ^{125}ASW and were washed and measured as above.

Sodium-independent iodine uptake mechanisms

In order to examine whether PDD is essential for larval iodine acquisition we exposed larvae to H_2O_2, reducing agents, peroxidase inhibitors, a metabolic inhibitor and an anion channel inhibitor. This addressed several components of the PDD model. Firstly, the effect of additional H_2O_2 was examined to determine whether the I^-+H_2O_2 reaction is involved in *S. purpuratus* iodine uptake. Secondly, exposing larvae to reducing agents that scavenge this ROS molecule assessed whether H_2O_2 is essential for larval iodine uptake. Thirdly, in the PDD model proposed by Amachi and colleagues, a peroxidase is needed to catalyze the I^-+H_2O_2 reaction (Amachi et al., 2007). Therefore, if this model applies to sea urchin larvae, iodide and H_2O_2 must react with a peroxidase, which was tested through the use of peroxidase inhibitors. The final part of this model is that iodine must diffuse into the organism through a channel and appropriate chemical inhibitors would block this.

Eggs of *S. purpuratus* release ~32 μmol l^{-1} of H_2O_2 during fertilization (Foerder et al., 1978) and we tested several H_2O_2 (Fisher) concentrations within this range. The effects of ascorbate (Sigma, St Louis, MO, USA), catalase (Sigma), aminotriazole (Sigma) and thiourea (Sigma) on iodide influx were examined. We also tested the effects of 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) and the metabolic inhibitor cyanide (both obtained from Sigma).

To further test the role of H_2O_2 in larval iodine influx, we induced oxidative stress using the insecticide Paraquat (Ultra Scientific, Kingston, RI, USA). Larvae were pre-incubated for 30 min in ASW containing one of five treatments: 0 mmol l^{-1} Paraquat (control), 0.1 mmol l^{-1} Paraquat, 1 mmol l^{-1} Paraquat, 10 mmol l^{-1} Paraquat and 10 mmol l^{-1} Paraquat + 100 μmol l^{-1} ascorbate (rescue). They were then exposed to ^{125}ASW for 5 min to investigate Paraquat's effect on initial iodine influx rate.

Sodium-dependent iodine uptake mechanisms

The effect of potassium perchlorate ($KClO_4$) was tested at three concentrations: 1, 10 and 100 μmol l^{-1}. Larvae were pre-incubated in ASW+$KClO_4$ (Sigma) for 10 min prior to the addition of ^{125}ASW. Furthermore, we assessed the effect of decreasing sodium availability by replacing it with the osmolyte *N*-methyl-D-glucamine (NMDG, Acros Organics, Fair Lawn, NJ, USA), but osmolality was maintained at 1000 mosmol l^{-1} (as measured by a Vapro vapor pressure osmometer, Wescor Biomedical Systems, UT, USA). The seawater consisted of: 486 μmol l^{-1} NaCl and/or NMDG+HCl (pH 8), 10 μmol l^{-1} KCl, 30 μmol l^{-1} $MgSO_4$, 26 μmol l^{-1} MgCl, 2.5 μmol l^{-1} $KHCO_3$, 10 μmol l^{-1} $CaCl_2$, 10 μmol l^{-1} Hepes, and 1 μmol l^{-1} EDTA (Beltrán et al., 1996). Five sodium concentrations were used: 467 μmol l^{-1} (control), 431.98 μmol l^{-1}, 396.95 μmol l^{-1}, 361.93 μmol l^{-1} and 326.9 μmol l^{-1}. Larvae were incubated in ^{125}ASW for 2.5 min, without pre-incubation. A second experiment was conducted in ASW without radioactive iodine to determine the effect of low sodium environments on survivorship. The effect of

Table 1. Putative orthologs for members of SSF across several vertebrate and invertebrate species

Bf-XP_002587551.1	Bf-XP_002587552.1	Bf-XP_002588189.1	Bf-XP_002589090.1
Bf-XP_002592457.1	Bf-XP_002594569.1	Bf-XP_002595839.1	Bf-XP_002595841.1
Bf-XP_002595848.1	Bf-XP_002598486.1	Bf-XP_002598597.1	Bf-XP_002602405.1
Bf-XP_002603317.1	Bf-XP_002605357.1	Bf-XP_002610578.1	Bf-XP_002612221.1
Bt-XP_002688618.1	Ci-XP_002119499.1	Ci-XP_002119750.1	Ci-XP_002119840.1
Ci-XP_002119869.1	Ci-XP_002120003.1	Ci-XP_002120451.1	Ci-XP_002120669.1
Ci-XP_002120797.1	Ci-XP_002120951.1	Ci-XP_002121367.1	Ci-XP_002121431.1
Ci-XP_002121552.1	Ci-XP_002122377.1	Ci-XP_002123007.1	Ci-XP_002124899.1
Ci-XP_002124996.1	Ci-XP_002125485.1	Ci-XP_002125925.1	Ci-XP_002127459.1
Ci-XP_002127459.1	Ci-XP_002128819.1	Ci-XP_002130613.1	Ci-XP_002130755.1
Ci-XP_002131513.1	Ci-XP_002131980.1	Ci-XP_002121226.1	Cl-XP_003432945.1
Dm-AAL90311.1	Dm-AAL90332.1	Dm-ADI46804.1	Dm-NP_572397.1
Dm-NP_609192.1	Dm-NP_611465.2	Dm-NP_647678.3	Dm-NP_650743.1
Dm-NP_651447.2	Dm-NP_651889.3	Dm-NP_651890.1	Dm-NP_727460.1
Dr-NP_001030141.1	Dr-NP_001082860.1	Dr-NP_956662.1	Dr-NP_998091.1
Dr-XP_001344597.1	Gg-XP_429095.3	Hs-AAI31543.1	Hs-NP_000444.1
Hs-NP_068587.1	Hs-NP_666018.3	Hs-NP_848593.2	Mm-NP_001171095.1
Mm-NP_071308.2	Mm-NP_444478.2	Mm-NP_573517.1	Nv-XP_001635645.1
Nv-XP_001636896.1	Nv-XP_001638262.1	Nv-XP_001638263.1	Nv-XP_001638621.1
Pt-XP_524154.2	Rn-NP_443215.2	Sk-NP_001161572.2	Sk-XP_002730902.1
Sk-XP_002731879.1	Sk-XP_002734548.1	Sk-XP_002735543.1	Sk-XP_002736421.1
Sk-XP_002736596.1	Sk-XP_002739433.1	Sk-XP_002739554.1	Sp-XP_001176711.1
Sp-XP_001194042.1	Sp-XP_003723535.1	Sp-XP_003724558.1	Sp-XP_003727266.1
Sp-XP_003728609.1	Sp-XP_780439.3	Sp-XP_781357.3	Sp-XP_781566.3
Sp-XP_783256.3	Sp-XP_785969.2	Sp-XP_785969.2	Sp-XP_785969.2
Sp-XP_787078.2	Sp-XP_789022.2	Sp-XP_793655.3	Sp-XP_795278.2
Sp-XP_795483.3	Sp-XP_797314.3	Sp-XP_797570.2	Sp-XP_797860.3
Sp-XP_798952.3			

The information in the table is given in alphabetical order (from left to right).
SSF5, sodium/solute symporter family 5.

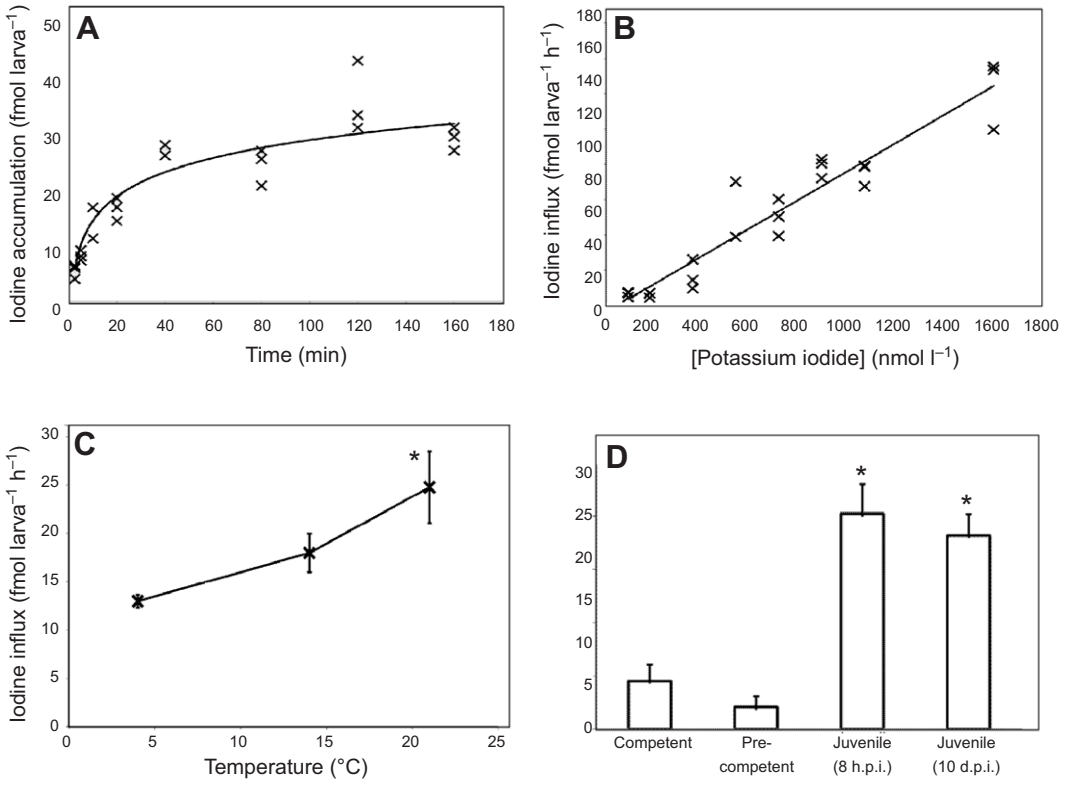

Fig. 1. Iodine influx for six-arm stage larvae of *Strongylocentrotus purpuratus* in artificial seawater (*N*=3). (A) The initial influx at 2000 nmol l^{-1} is apparent by ~2.5 min and reaches a plateau at ~40 min. (B) Increasing potassium iodide concentration does not decrease iodine influx rate during 2.5 min incubation. (C) There is a positive correlation between temperature and iodine influx rate in six-arm stage larvae (*significant increase in influx rate compared with 4°C treated larvae at P<0.05; Fisher's LSD). (D) Influx rate during a 30 min incubation is affected by the developmental progression of metamorphosis. Late stage competent (not induced to metamorphose) and pre-competent (induced to metamorphose) larvae have significantly lower iodine influx rates than metamorphosed individuals (induced to metamorphose) (*N*=3). *Significant increase in iodine influx compared with late stage competent larvae. h.p.i., hours post-induction; d.p.i. days post-induction.

the medium on mortality was assessed through observation of mobility, muscle contraction and cilliary beating under a dissecting microscope.

Statistical analysis

Influx rate (fmol larvae^{-1} h^{-1}) was calculated by Eqn 1 (modified from Chang et al., 1997):

$$J_{in} = \frac{Q_{larvae}}{X_{out} \cdot t_{inc}}\ , \quad (1)$$

where J_{in} is the net influx of iodine (fmol individual^{-1} h^{-1}), Q_{larvae} is the radioactivity of larvae or eggs (c.p.m. individual^{-1}), X_{out} is the specific activity of the incubation water (c.p.m. fmol^{-1} I^{-}) and t_{inc} is the incubation time (h).

Pharmacological results were analyzed using SPSS (www.ibm.com/software/analytics/spss). The effects of various inhibitors on iodine influx were analyzed using ANOVA with a Fisher's LSD *post hoc* test after an initial test for normal distribution using Shapiro–Wilk test for normality. This is with the exception of DIDS, where a *t*-test was used. Regression analysis was conducted on data testing the effect of KI concentration on influx rate.

Phylogenetic analysis of SSF5

Human protein sequences were used in a basic local alignment search tool (BLAST) search for putative orthologs for members of SSF5, specifically a tblastn with an e-value cut off at 1 e–04. These BLAST searches were carried out on the NCBI site (www.ncbi.nlm.nih.gov/) for orthologs of the human sodium–glucose transporter 2 (SLC5A2), NIS (SLC5A5), choline transporter (CHT; SLC5A7), AIT (SLC5A8) and the sodium-coupled monocarboxylate transporter 2 (SMCT; SLC5A12). Representative sequences were obtained for: *Homo sapiens* (Hs), *Danio rerio* (Dr), *Branchiostoma florididae* (Bf), *Ciona intestinalis* (Ci), *Strongylocentrotus purpuratus* (Sp), *Saccoglossus kowalevski* (Sk), *Drosophila melanogaster* (Dm), *Nematostella vectensis* (Nv), *Pan troglodytes* (Pt), *Canis lupus* (Cl), *Mus musculus* (Mm), *Rattus norvegicus* (Rn), *Bos taurus* (Bt) and *Gallus gallus* (Gg). For the full list of the 109 sequences used, see Table 1. These sequences were then aligned in SeaView and a PhylMyl tree was created using an LG matrix (Gouy et al., 2010). An alignment of vertebrate NIS sequences and a selection of sequences from *S. purpuratus* can be found in supplementary material Fig. S1.

RESULTS

Kinetics of iodine uptake in *S. purpuratus*

Incubation in ^{125}ASW demonstrated that larvae of *S. purpuratus* acquire iodide directly from the environment in the absence of food (Fig. 1A). When larvae were incubated in ASW containing 2000 nmol l^{-1} potassium iodide (KI), the iodide accumulation plateaued after 40 min and the initial influx rate (where the accumulation is linear) occurred within the first 10 min at this iodine concentration. We also found that with increasing iodine concentration (100–1800 nmol l^{-1} KI) the influx rate did not decrease (adjusted R^2=0.709, P<0.001, Fig. 1B). Furthermore, this influx followed a linear pattern within the environmentally relevant range (100–800 nmol l^{-1} KI).

The effects of pH and temperature were tested in order to assess additional base parameters of the uptake process. For example, temperature dependence indicates the involvement of a metabolic process. Influx that is dependent on pH suggests that a specific oxidation form of iodide is translocated across the membrane as this abiotic variable is known to influence marine iodine chemistry. Temperature had a significant impact on iodide influx ($F_{3,3}$=5.79, P<0.05), where influx at 21°C was significantly higher than that at 4°C (Fig. 1C). Across the range tested, pH had a non-significant effect on iodine influx ($F_{3,3}$=3.46, P>0.05).

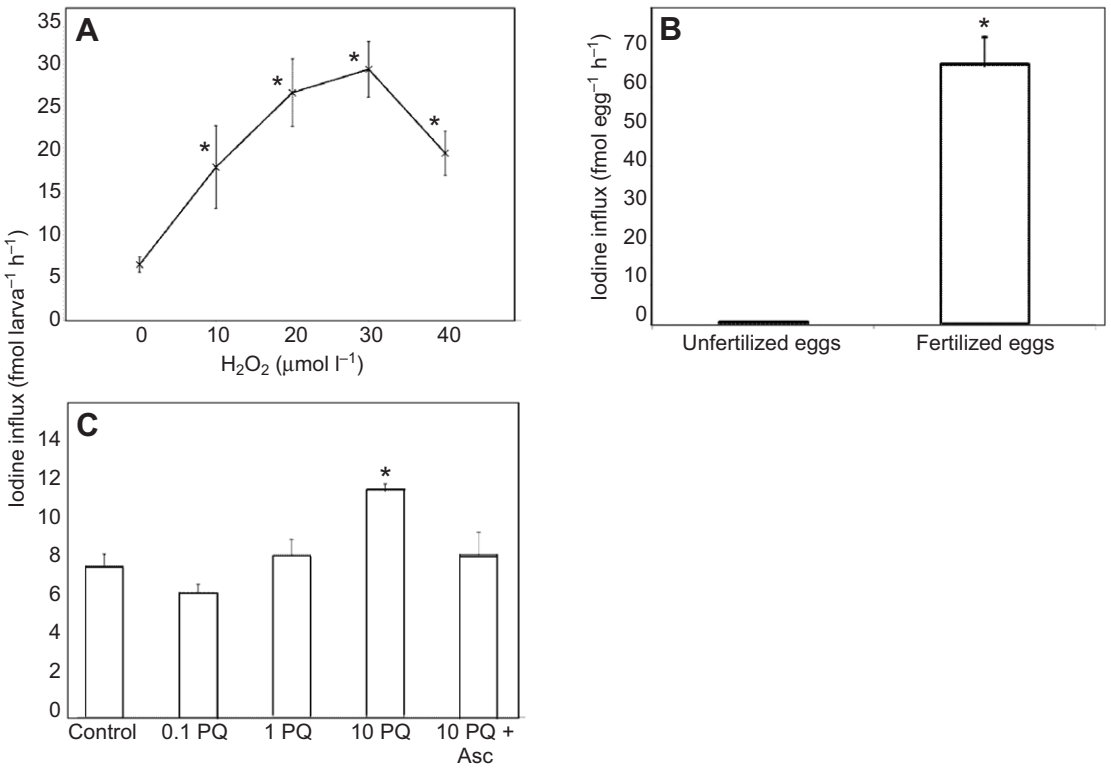

Fig. 2. (A) Exogenous iodine uptake rate is enhanced significantly by the addition of exogenous hydrogen peroxide (H_2O_2) in six-arm stage larvae of *S. purpuratus* (*N*=4). (B) The oxidative burst at fertilization is associated with an increase in iodine influx relative to unfertilized eggs (*N*=3). (C) Paraquat (PQ) exposure was found to increase iodine influx in six-arm stage larvae at the 10 mmol l^{-1} concentration (10 PQ). This effect was rescued by addition of 10 μmol l^{-1} ascorbate (10 PQ + Asc) (*N*=3). *Significant difference at the *P*<0.05 level from the control (Fisher's LSD).

There appeared to be a significant effect of metamorphosis on iodine influx in sea urchins ($F_{3,2}$=10.67, *P*<0.05; Fig. 1D). Biofilm exposure alone did not affect iodine uptake as individuals that were induced with biofilm but did not metamorphose exhibited the same influx rates as competent un-induced individuals. Metamorphosis, specifically, caused the increase in iodine influx; this was apparent based on the similarity between iodine influx rates in juveniles 8 h post-induction and those of juveniles 10 days post-induction (Fig. 1D).

Sodium-independent iodine uptake mechanisms

We found that the initial iodine influx rates of six-arm stage larvae increase linearly with the addition of exogenous hydrogen peroxide from 0 to 30 μmol l^{-1} with a non-significant decrease at 40 μmol l^{-1} in 100 nmol l^{-1} iodine environment ($F_{4,3}$=7.22, *P*<0.005; Fig. 2A). Similarly, we were able to demonstrate a significant increase in iodide influx associated with fertilization in sea urchin eggs ($F_{3,3}$=31.26, *P*<0.001, Fisher's LSD, *P*<0.001; Fig. 2B).

Iodine incorporation at fertilization was inhibited by the addition of 100 μmol l^{-1} ascorbate, a reducing agent that scavenges available hydrogen peroxide ($F_{3,3}$=31.26, Fisher's LSD, *P*<0.001; Table 2). When six-arm stage larvae were exposed to an identical treatment, there was also a decrease in initial iodine influx rates, though not as pronounced as in fertilized eggs ($F_{4,3}$=6.575, *P*<0.05, Fisher's LSD; Table 2). We found that exposure to catalase, another consumer of hydrogen peroxide, also led to a reduction of iodine influx rates in larvae ($F_{4,3}$=6.575, *P*<0.05, Fisher's LSD, *P*<0.05), but not in eggs ($F_{3,3}$=31.26, Fisher's LSD, *P*>0.05; Table 2).

Paraquat induces oxidative stress through the production of superoxide by redox cycling *in vivo* (Bus and Gibson, 1984). This induced oxidative stress in larvae resulted in a significant increase in the initial influx rate in six-arm stage larvae ($F_{4,3}$=6.06, *P*<0.01; Fig. 2C). However, when 100 μmol l^{-1} ascorbate was added to the Paraquat treatment, the influx rate was not significantly different to the control (Fisher's LSD, *P*>0.05; Fig. 2C).

Table 2. Effects of various inhibitors on initial iodine influx rates in six-arm stage *S. purpuratus* larvae and in eggs

		Uptake activity (% of control)	
Additive	Concentration	Six-arm stage larvae	Fertilized eggs
Perchlorate	1 μmol l^{-1}	95.5	
	10 μmol l^{-1}	90.2	
	100 μmol l^{-1}	74.6	
Aminotriazole	10 μmol l^{-1}	56.9*	43.8*
	100 μmol l^{-1}	56.0*	33.1*
	1000 μmol l^{-1}	49.7*	30.5*
Thiourea	1 nmol l^{-1}	76.6	67.7*
	100 nmol l^{-1}	45.9*	26.9*
	1000 nmol l^{-1}	9.7*	0.77*
Cyanide	1 μmol l^{-1}	100.0	26†
	100 μmol l^{-1}	49.5*	5†
	1000 μmol l^{-1}	9.5*	0†
Catalase	10 U ml^{-1}	79.9	100.0
	100 U ml^{-1}	49.9*	
	1000 U ml^{-1}	36.9*	
Ascorbate	100 μmol l^{-1}	61.6*	1.94*
DIDS	0.1 μmol l^{-1}	39.5*	

*Significantly different from the control at the *P*<0.05 confidence level.
†Data taken from Klebanoff et al. (Klebanoff et al., 1979).

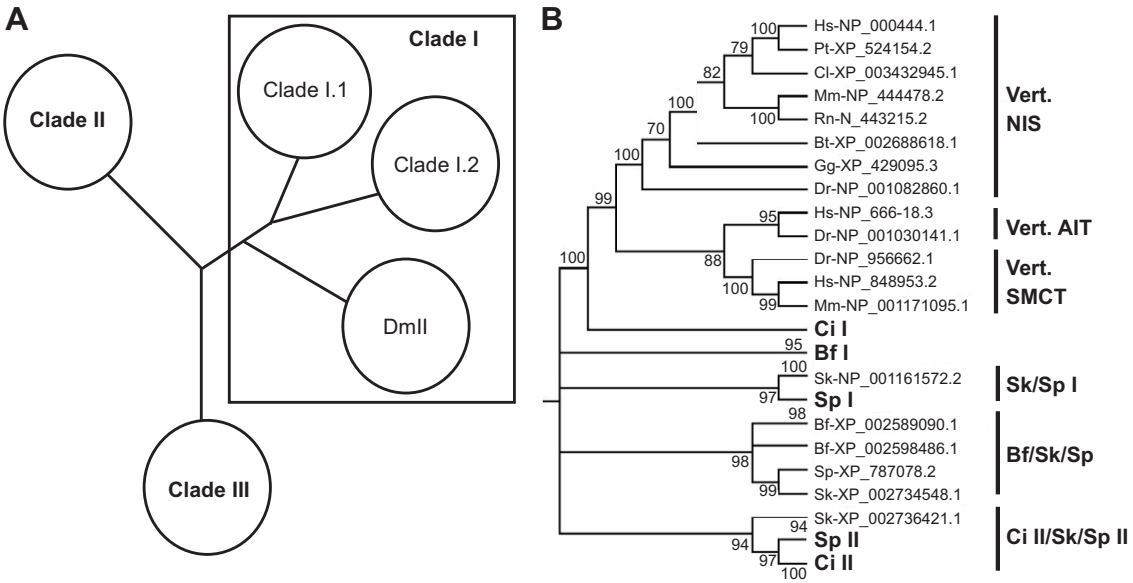

Fig. 3. (A) Schematic topology of the sodium/solute symporter family 5 (SSF5) using several vertebrate and invertebrate taxa. Based on an Amino LG matrix, there are three major clades (supported with a bootstrap level of 100%): Clade I, Na/anion transporter-like proteins; Clade II, Na/glucose transporter-like proteins; and Clade III, choline transporter-like proteins (CHT). Clade I contains Clade I.1 sodium/iodide symporter (NIS)/apical iodide transporter (AIT)/sodium-coupled monocarboxylate transporter (SMCT)-like proteins, Clade I.2 invertebrate Na/anion transporter-like proteins and Dm II *Drosophila* Na/anion transporter-like proteins. (B) Clade I.1 contains the vertebrate NIS, AIT and SMCT proteins and deuterostome representatives of the SSF5. All vertebrate sequences cluster together and the sister group to the vertebrate clade is Ci I, which contains eight *Ciona* representatives. The remaining four clusters are unresolved at the 60% bootstrap level: Bf I, Sk/Sp I, Bf/Sk/Sp and Ci II/Sk/Sp II. Bf I, a cluster of eight *Branchiostoma* proteins is supported by 100% bootstrap. Sp I contains five proteins from the sea urchin. Sp II contains six proteins and Ci II contains seven proteins. Vert., vertebrate. For other species abbreviations, see Fig. 4.

As previously mentioned, the reaction between iodide and H_2O_2 generally requires a peroxidase catalyst (Luther et al., 1995). We therefore examined the effects of thiourea and aminotriazole, two peroxidase inhibitors (Wood and Legg, 1970; Davidson et al., 1979), on iodide accumulation. We found a statistically significant decrease in larval iodine influx with increasing thiourea concentration (from 1 to 1000 nmol l^{-1}) during a 30 min incubation ($F_{4,2}$=6.16, P<0.05; Table 2). Fertilized eggs exposed to thiourea experienced a dose-dependent decrease in iodine influx as was observed in larvae ($F_{5,2}$=147.65, P<0.001; Table 2). Aminotriazole significantly disrupted the initial iodide influx in six-arm stage larvae ($F_{3,3}$=6.88, P<0.01; Table 2).

In order to test the energy dependence of iodine uptake, we exposed larvae at the six-arm stage to cyanide and found that cyanide was a strong inhibitor of iodine influx with increasing concentrations ($F_{3,3}$=13.226, P<0.01; Table 2). To test the involvement of anion channels in the uptake process, we exposed six-arm stage larvae to DIDS and found a significant decrease in iodine influx (t_3=5.2, P<0.01, Table 2).

Sodium-dependent iodine uptake mechanisms

Decreasing sodium availability in the environment had no impact on iodine influx in six-arm stage larvae ($F_{4,3}$=2.12, P>0.05). Furthermore, larvae exposed to potassium perchlorate, an inhibitor of NIS/AIT transport, did not experience any inhibition of iodine influx rate ($F_{3,3}$=1.163 P>0.05, Table 2). There was no permanent effect of exposure to reduced sodium seawater on larval motility and survivorship (results not shown).

Phylogenetic analysis of the SSF5

Our phylogeny based on an Amino LG matrix determined that, for the 109 proteins included in this analysis, there are three major clades, each supported with a bootstrap value of 100% (Fig. 3A). There was one *Nematostella* sequence outside of these three major clades (Nv-XP_001621852.1) and it was not included in Fig. 3A, but is shown in Fig. 4. Clade I, the Na/anion transporter-like proteins, are a group of vertebrate and invertebrate proteins related to the vertebrate NIS, AIT and SMCT. Clade II, Na/glucose transporter-like proteins, cluster around SLC5A2, the vertebrate sodium/glucose transporter 2. Clade III, the choline transporter (CHT)-like proteins, contains several invertebrate representatives that group near the vertebrate CHT. Furthermore, Clade I contains Clade I.1 NIS/AIT/SMCT-like proteins. The vertebrate NIS, AIT and SMCT proteins are found within this group and the rest of this clade consists of only deuterostome representatives of the SSF5 (Fig. 3B).

The vertebrate sequences found in Clade I.1 form a monophyletic group (99% bootstrap support). The sister group to the vertebrate clade is Ci I (100% bootstrap support), which contains eight *Ciona* representatives. The remaining four clusters were unresolved at the 60% bootstrap level. They are Bf I, Sk/Sp I, Bf/Sk/Sp and Ci II/Sk/Sp II. Bf I, a cluster of eight *Branchiostoma* proteins is supported by 100% bootstrap (Fig. 4). Across the phylogeny, *Saccoglossus* proteins tend to cluster with sequences from *Strongylocentrotus*. Here, we find in Sk/Sp I that one *Saccoglossus* protein groups with Sp I, a cluster of five sea urchin proteins. There is a small protein cluster, Bf/Sk/Sp, supported at the 98% bootstrap level and containing two *Branchiostoma* sequences, one *Strongylocentrotus* sequence and one *Saccoglossus* sequence. Finally, the last clade, Ci II/Sk/Sp II, consists of a *Saccoglossus* protein grouped with a cluster containing Sp II (six proteins) and Ci II (seven proteins). See Fig. 4 for the accession numbers of the aforementioned proteins.

Outside of Clade I.1, in the Na/anion transporter-like proteins group, the non-vertebrate proteins are clustered into two groups.

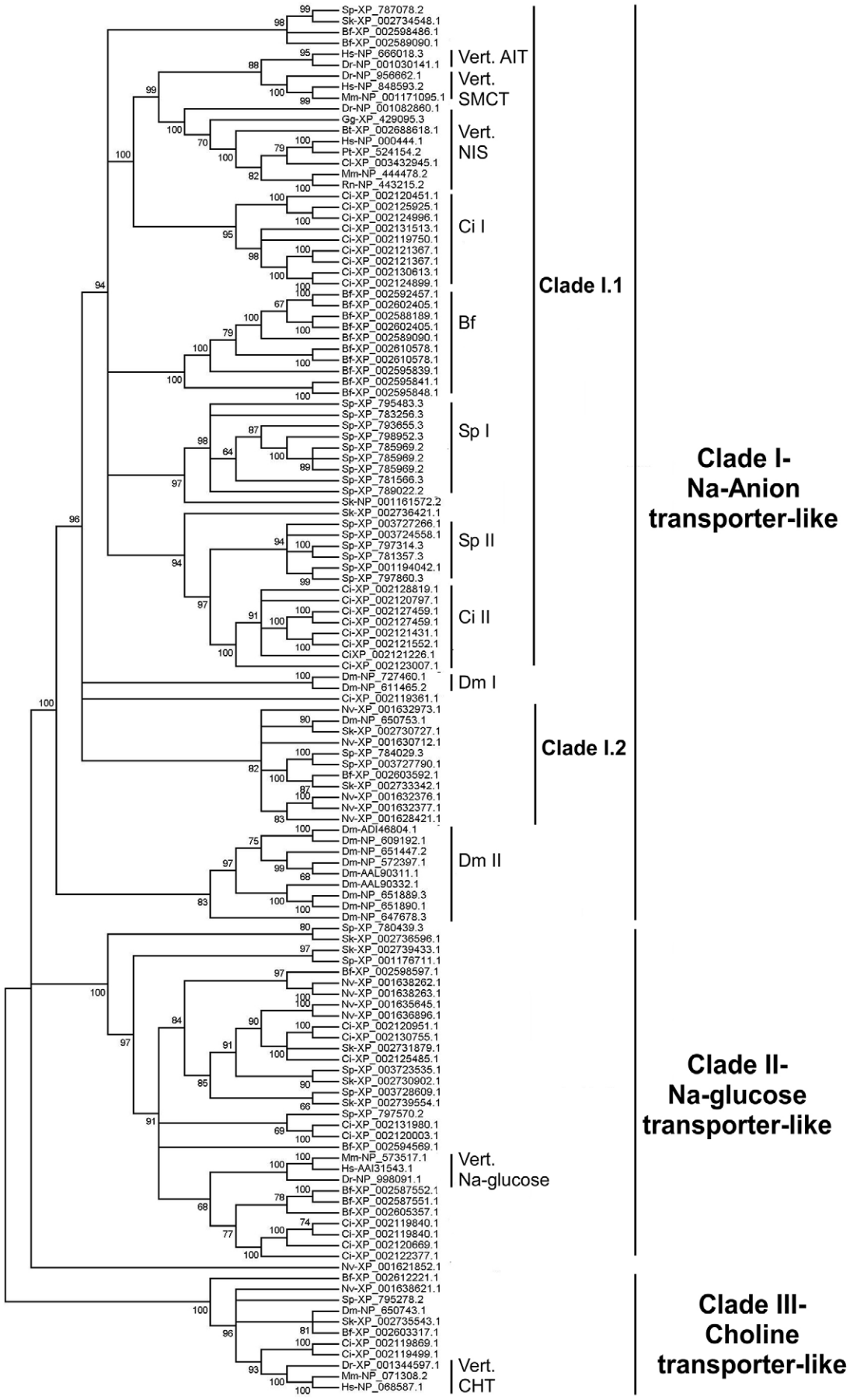

Fig. 4. A PhyML tree of amino acid sequences found for members and orthologs of the sodium/solute symporter family 5 (SSF5), based on an Amino LG matrix. Bootstrap values were cut off at 60%. In total, 109 sequences were examined for *Homo sapiens* (Hs), *Pan troglodytes* (Pt), *Canis lupus* (Cl), *Bos taurus* (Bt), *Mus musculus* (Mm), *Rattus norvegicus* (Rn), *Gallus gallus* (Gg), *Danio rerio* (Dr), *Branchiostoma floridae* (Bf), *Ciona intestinalis* (Ci), *Strongylocentrotus purpuratus* (Sp), *Saccoglossus kowalevski* (Sk), *Drosophila melanogaster* (Dm) and *Nematostella vectensis* (Nv). CHT, choline transporter.

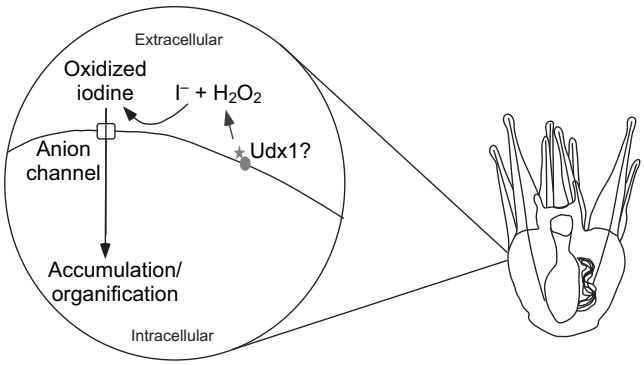

Fig. 5. Hypothesized mechanism of iodine influx in sea urchin larvae based on the peroxide-dependent diffusion (PDD) model. In this model, iodine reacts with hydrogen peroxide (H_2O_2) through the action of a peroxidase. We hypothesize that the sea urchin NADPH oxidase dual oxidase 1 (Udx1) fulfils this role. Oxidized iodine then moves through a channel where it is accumulated and/or orgnanified intracellularly.

The first, Clade I.2, which consists of representatives from all of the taxa under examination, with the exception of vertebrates, was supported by 96% bootstrap. Sister to this is Dm II, a clade containing 10 sequences from *Drosophila* (Fig. 4).

DISCUSSION

Iodine uptake has not been studied in detail for non-chordate animals despite the fact that iodine uptake, function and metabolism have been described across a wide range of taxa (reviewed by Eales, 1997; Miller and Heyland, 2010) (see also Heyland et al., 2005). In contrast to the relatively well-described sodium transporter-dependent iodine uptake mechanisms in chordates, recent studies suggest that sodium transporter-independent mechanisms such as PDD are crucially important for iodine uptake and regulation in macroalgae, bacteria and microalgae (Küpper et al., 1998; Amachi et al., 2007) (S. van Bergjik, personal communication). Here, we investigated iodine uptake mechanisms in larvae of the purple sea urchin *S. purpuratus* and tested whether iodine uptake in planktonic larvae of this species is dependent on a transporter system such as the NIS or is diffusion based and dependent on peroxide.

Initial iodine influx rates in sea urchin larvae suggest a diffusion-based mechanism for iodine accumulation

Iodine concentration and its chemical composition in seawater (iodide and iodate being the most common) vary significantly between habitats and geographical regions. For example, while average total inorganic iodine is 400–500 nmol l^{-1} (Fuge and Johnson, 1986), iodide in marine surface waters ranges from <62 nmol l^{-1} near the poles to 230 nmol l^{-1} in tropical and subtropical environments (Wong, 1991). Kinetic data from our radioactive iodine accumulation assays strongly indicate that iodine influx is not likely to be transporter dependent, as the initial iodine influx rate did not saturate with increasing iodine concentrations within the range 100–1800 nmol l^{-1} iodine. Furthermore, sea urchin larvae accumulate iodine at concentrations well above natural levels in what is presumably their habitat as planktotrophic larvae (i.e. surface waters of the Pacific) before they become competent and settle into their adult habitat. In surface water, iodine occurs at ~78 nmol l^{-1} (Tsunogai, 1971). As the larval uptake capacity greatly exceeds the availability of unbound iodine, sea urchin larvae are able to efficiently accumulate iodine regardless of its environmental availability.

This is similar to what was observed in Atlantic halibut (*Hippoglossus hippoglossus*). Here, the accumulation of iodide is uninhibited by increasing environmental concentrations, such that even in media containing excessive iodine (much higher than would be present in the natural environment), the larval influx does not plateau (Moren et al., 2008). NIS seems to be involved in this process; however, as only half of the iodide uptake is inhibited by perchlorate, perchlorate-independent uptake through ingestion was proposed for early stages lacking functional gills (Moren et al., 2008). For these larvae, dietary sources of iodine seem to be crucial based on the following evidence: the high iodine concentrations in their copepod prey (estimated to be on average 94 mmol l^{-1}) (Moren et al., 2006), their extensive absorption capacity to take up iodide from seawater (Moren et al., 2008), and the positive effect dietary iodine has on their development and survivorship (Hamre et al., 2002).

This information raises the general question about how important food sources are for providing iodine to aquatic organisms. Planktonic invertebrate larvae, including feeding larvae of the purple sea urchin, spend a considerable amount of time in the water column feeding on microalgae. Previous work including recent data from our lab has demonstrated that microalgae species that larvae feed on have the capacity to accumulate iodine and potentially metabolize it to other organic forms such as thyroid hormones (Chino et al., 1994; Heyland et al., 2006) (S. van Bergjik, personal communication). By feeding on algae, sea urchin larvae may receive iodine in a much more concentrated form. Furthermore, if larvae receive TH precursors or the active hormones themselves, these can affect their metabolism during development and metamorphosis (Chino et al., 1994; Heyland, 2004; Heyland et al., 2004; Heyland and Moroz, 2005; Heyland and Moroz, 2006; Heyland et al., 2006).

Cyanide acts by interrupting the electron transport chain (by blocking cytochrome oxidase activity) and by limiting the availability of ATP (Gensemer, 2006). Unlike the uptake system observed in marine bacteria, the metabolic inhibitor cyanide significantly disrupts sea urchin iodide influx. In bacteria, a 1 mmol l^{-1} treatment results in a ~30% decrease in iodide influx (Amachi et al., 2007), while in sea urchin larvae this cyanide concentration results in a >90% reduction in iodide uptake. While this is not an ultimate test of whether this process is active or passive, it is a good indicator of energy dependence. Therefore, we conclude that although iodide uptake in sea urchin larvae is largely through a transporter-independent mechanism it does appear to require ATP.

It is apparent that iodide influx is significantly higher post-metamorphosis regardless of whether the larvae have been treated with biofilm and irrespective of how long the individual has lived as a juvenile (8 h post-induction *versus* 10 days post-induction). This indicates that juveniles have a significantly higher iodide uptake capacity than late-stage larvae of comparable size. Changes in transport kinetics as a result of developmental transitions have been documented before in *S. purpuratus* with regards to integumental uptake of dissolved organic carbon (Allemand et al., 1984; Davis et al., 1985) (reviewed in Wright and Manahan, 1989). However, how the increase in iodine uptake rate with metamorphosis relates to life history transitions and the subsequent changes in environment (i.e. movement from the pelagic to the benthic environment) in *S. purpuratus* is unclear, as iodine concentration is lower in marine surface water (0–200 m) and increases with depth (>200 m)

(Truesdale, 1978). Future studies tracing iodine in larval and metamorphic stages may be useful in determining whether changes in iodine metabolism can explain the differences in uptake rate.

Hydrogen peroxide affects iodine influx in sea urchin larvae

While NIS/AIT-dependent iodide uptake has been best characterized in the vertebrate thyroid gland, it is not the only known mechanism responsible for iodine uptake. PDD has recently been described in some detail for both bacteria and macroalgae. In a marine Flavobacteriaceae bacteria strain (Amachi et al., 2007) and *Laminaria* kelp (Küpper et al., 1998), peroxide oxidizes iodine *via* an as yet unidentified haloperoxidase; hypoiodous acid (HIO) is speculated to be the form of iodine produced by this system (Amachi et al., 2007). For Flavobacteriaceae bacteria it has been further shown that the peroxide released into the extracellular environment originates from glucose oxidase activity in the cell membrane (Amachi et al., 2007).

Our data strongly suggest that a peroxide-dependent mechanism is at least partially involved in iodine uptake in sea urchin larvae. This conclusion is substantiated by several experimental findings from our study. When larvae were exposed to peroxide and Paraquat (a producer of intracellular peroxide), iodine accumulation increased significantly (Bus and Gibson, 1984). We were also able to demonstrate that iodine accumulation is inhibited by treatment of larvae with hydrogen peroxide scavengers (ascorbate and catalase) and peroxidase inhibitors (thiourea and aminotriazole). Furthermore, while we examined cyanide activity in the context of metabolism, this chemical also has inhibitory effects on some peroxidases (e.g. Klebanoff et al., 1979). These pharmacological data indicate that hydrogen peroxide is produced by larval tissue and is crucial for iodine uptake. Furthermore, in normal development, sea urchin embryos experience an oxidative burst that is associated with a sudden release of hydrogen peroxide at fertilization. This ultimately leads to the formation of the fertilization envelope, through ovoperoxidase-mediated tyrosine cross-linking and acts as a slow block to polyspermy (Foerder et al., 1978; Wong et al., 2004; Wong and Wessel, 2005). Our data confirm that this release of H_2O_2 is also associated with a substantial increase of iodine accumulation.

The enzyme responsible for the oxidative burst at fertilization is Udx1, a member of the NADPH oxidases, which are characterized by a broad set of functions including ROS metabolism and TH synthesis (Wong et al., 2004); these enzymes are diphenyleneiodonium sensitive (Cross and Jones, 1986) but cyanide insensitive (Babior et al., 1976). In sea urchins, Udx1 protein is expressed in the ectodermal domain of the embryo and has also been shown to be expressed in later embryonic stages (Wong and Wessel, 2005). Pharmacological and functional-blocking experiments of sea urchin Udx1 have demonstrated that hydrogen peroxide produced by Udx1 appears to be necessary for the early cell cycle (Wong and Wessel, 2005). Our results show that iodine uptake increases dramatically at fertilization. These data therefore provide a strong natural test of the hypothesis that peroxide facilitates the uptake of iodine. Based on this evidence, we propose a new model for iodine uptake in sea urchin embryos and larvae (Fig. 5), suggesting that Udx1 may produce the peroxide essential for iodine accumulation.

In our model, the oxidation of iodide is essential to its ability to be taken up by cells. While we have no information regarding the specific form of iodine taken up by sea urchins, we hypothesize that such a compound could be hypoiodous acid (HIO) or some oxidation product of it. When molecular iodine (I_2) is formed in seawater through oxidation, a major part of it disproportionates into HIO (Truesdale, 1993). It has also been proposed that HIO is the form that can enter the cells in Flavobacteriaceae (Amachi et al., 2007). Based on this evidence it would be important in future studies to further investigate the chemical properties of iodide oxidation products that are involved in the uptake process.

Note, however, that the oxidative burst in sea urchin fertilization is different to the oxidative burst that occurs in *Laminaria*, where it functions in microbial defense (Küpper et al., 2001; Küpper et al., 2002; Küpper et al., 2006). There, iodide stored in the apoplast acts as an extracellular antioxidant as natural antioxidants, such as ascorbate and glutathione, used by *Laminaria* are found strictly intracellularly (Küpper et al., 2008). Studies also showed that in contrast to the sea urchin oxidative burst, oxidative stress (e.g. photo-oxidative ROS production) or an oxidative burst in *Laminaria* results in an efflux of iodide (Küpper et al., 2008). Within the apoplast [where H_2O_2 concentrations can reach an excess of 1 mmol l^{-1} (Küpper et al., 2001)] and the thallus surface, released iodide acts as an antioxidant and scavenges ROS (Küpper et al., 2008).

While the system described by Küpper and colleagues (Küpper et al., 2008) characterizes a novel mechanism for iodine storage and use, a model for iodine antioxidant function proposed for the moon jelly (*Aurelia aurita*) (Berking et al., 2005) is more relevant to the system we propose for sea urchin larvae. For *A. aurita*, Berking and colleagues hypothesized that iodide and tyrosine synergistically function as a non-enzymatic ROS defense system (Berking et al., 2005). They propose that iodide enters *A. aurita* tissue (through an as yet uncharacterized mechanism) where it reacts with endogenous ROS to form iodine. Iodine then reacts with tyrosine and produces iodotyrosines, which form a waste product that can diffuse out of the tissue or, as the authors speculate, become co-opted as a signaling molecule (Berking et al., 2005). This proposed mechanism is equivalent to the biochemical process occurring during the oxidative burst at fertilization where hydrogen peroxide catalyzes the cross-linking of tyrosine to harden the fertilization membrane (Foerder et al., 1978; Wong et al., 2004; Wong and Wessel, 2005). As sea urchins possess the necessary cellular machinery for this process, it is plausible that this mechanism is also employed in antioxidant function. Furthermore, it is conceivable that these enzymes (e.g. Udx1) are also used during later development for iodine uptake or in iodine metabolism such as the iodination of biological molecules.

Sea urchin iodine influx is not dependent on NIS or AIT orthologs

Based on current evidence, understanding of sodium-dependent iodine uptake mechanisms is restricted to the NIS and the AIT, which both occur in the vertebrate thyroid gland. Of these mechanisms, NIS is an active transporter whereas AIT is involved in the passive diffusion of iodide down its concentration gradient (Eskandari et al., 1997; Rodriguez et al., 2002). Beyond this, no other sodium-dependent iodine uptake mechanism has been characterized in plants or animals. In order to test whether iodine accumulation in sea urchin larvae is NIS/AIT dependent, we tested the effect of perchlorate, a competitive inhibitor of NIS and AIT (Smanik et al., 1996; Rodriguez et al., 2002), on iodide accumulation in sea urchin larvae. We also tested the effect of decreased sodium concentrations in the environment on iodine accumulation. Finally, we searched the sea urchin genome (Sodergren et al., 2006) for NIS/AIT candidate genes.

Perchlorate is a potent inhibitor of iodide transport into the thyroid gland in vertebrates (Carrasco, 1993). This inhibition is established by a competition between iodide and perchlorate at a specific site of the NIS molecule (reviewed by Dohán and Carrasco, 2003). Our data show that perchlorate has no effect on iodine influx in sea urchin larvae, which is a very different result from what one would expect to see in vertebrates. The results therefore show that iodine accumulation is NIS/AIT independent and is also unaffected by exogenous sodium concentration within an environmentally relevant range. When we examined the effect of reduced sodium on survivorship, we found that larvae stopped swimming after 2.5 min in the lowest sodium concentration used; however, when they were transferred back into normal-sodium seawater, they recovered. This indicates that while sodium limitation does have an effect on larval physiology, it was not lethal for the duration of the pharmacology experiment (i.e. 2.5 min).

There are a few other examples of organisms that accumulate iodine in a way that is unaffected by perchlorate. For example, bryozoans (*Bugula neretina* and *Shizoporella errata*) accumulate radioiodide from seawater by ouabain- and dinitriphenol-sensitive uptake that is unaffected by perchlorate and also thiourea (reviewed in Eales, 1997). In scyphozoan jellyfish (cnidarians), where iodine is required for signaling developmental transitions (Spangenberg, 1974) and for use in a non-enzymatic antioxidant system (Berking et al., 2005), iodine uptake is also unaffected by perchlorate (Silverstone et al., 1978). Considering the vast phylogenetic distance between bryozoans, cnidarians and echinoderms, it appears that perchlorate-inhibited iodide uptake mechanisms, namely NIS/AIT, are more the exception than the rule among animals as a whole, and may even be vertebrate specific.

Evolutionary implications

NIS and AIT proteins are part of the SSF5. This family consists of at least 220 members ranging from bacteria to metazoa and, with a few exceptions, these proteins largely transport substrates (such as glucose, myoinositol and iodide) in a sodium-dependent manner (reviewed in Wright and Turk, 2004). In humans, there are currently 12 identified members expressed in a variety of tissues (Kanai et al., 1994; Jung, 2002). These proteins are highly conserved with up to 70% amino acid identity to SLC5A1 (sodium/glucose transporter 1) (Wright and Turk, 2004). It is because of this conservation that we analyzed representatives of this family across several vertebrate and invertebrate taxa in an attempt to identify putative *S. purpuratus* NIS/AIT orthologs.

As previously described (Wright and Turk, 2004), CHT-like proteins (Clade III), Na/glucose transporter-like proteins (Clade II) and Na/anion transporter-like proteins (containing NIS/AIT/SMCT; Clade I) are distinct phylogenetic clades. However, our analysis revealed that Clade I and Clade II group together and separately from Clade III (CHT-like proteins). These results are markedly different from what was found using only human protein sequences. Here, CHT grouped with NIS/AIT/SMCT as a separate cluster from the Na/glucose and Na/myoinositol transporters (Wright and Turk, 2004). Our results, using a wider selection of taxonomic groups including vertebrate and invertebrate sequences, indicate that the diversification of Na/glucose transporter-like proteins and Na/anion transporter-like proteins from a putative ancestral transporter occurred early in animal evolution.

With respect to the specific question addressed in this study, Clade I provides the most useful information. Specifically, we found that all NIS/AIT/SMCT relatives from invertebrate species are found only in deuterostomes (Clade I.1) and no protein sequences related to NIS can be found outside of the vertebrates (Clade I.1, Clade I.2 and Dm II). Therefore, we conclude that the NIS pathway, used for iodine uptake, is likely to be a vertebrate synapomorphy. These results contrast with the conclusions drawn by Paris and colleagues that *Branchiostoma* contains homologs of vertebrate NIS (Paris et al., 2008). While this group did conduct a thorough phylogenetic analysis, their phylogeny lacked sequences from *Saccoglossus* (a hemichordate), which may have contributed to the different conclusion.

In contrast to NIS/AIT mechanisms, the involvement of peroxidase activity in iodide accumulation is widespread, having been observed in macroalgae (Küpper et al., 1998), marine bacteria (Amachi et al., 2007) and microalgae species (S. van Bergjik, personal communication). Furthermore, iodine and peroxidases were present in primitive *Cyanobacteria* about 3.5 billion years ago (Obinger et al., 1997), indicating that the elements necessary for PDD as a mechanism for iodine uptake are very ancient. Whether this is the ancestral state for all organisms or whether it evolved many times independently in different lineages will require a more detailed mechanistic understanding of this process in a variety of organisms from different kingdoms.

Finally, it is worth noting that diffusion-based iodine uptake mechanisms may also function in aquatic vertebrates, e.g. teleosts. As mentioned previously, Moren and colleagues found that when halibut larvae were exposed to perchlorate, only half of the iodine influx was inhibited (Moren et al., 2008). Although PDD was never explicitly tested, it is conceivable that NIS/AIT-independent mechanisms of iodine acquisition may be more prevalent in vertebrates than originally assumed.

CONCLUSIONS

Our data show that sea urchins, a representative non-chordate deuterostome, utilize hydrogen peroxide-dependent diffusion for iodine acquisition. Putative orthologs of NIS/AIT transporters have been found in genomes of basal chordates but pharmacological evidence elucidating mechanisms of iodine uptake is largely missing from these taxa. Future work should focus on these mechanisms in such taxa as this will assist in our understanding of whether sodium-dependent iodine uptake is a vertebrate or chordate synapomorphy. Our preliminary analysis, based only on phylogenetic comparisons, suggests that this uptake mechanism is restricted to vertebrates.

LIST OF SYMBOLS AND ABBREVIATIONS

AIT	apical iodide transporter
ASW	artificial seawater
CHT	choline transporter
DPP	differential pulse polarography
FSW	filtered synthetic seawater
NIS	sodium iodide symporter
PDD	hydrogen peroxide-dependent diffusion
ROS	reactive oxygen species
SSF5	sodium/solute symporter family 5
THs	thyroid hormones

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REFERENCES

Aceves, C., Anguiano, B. and Delgado, G. (2005). Is iodine a gatekeeper of the integrity of the mammary gland? *J. Mammary Gland. Biol. Neoplasia* **10**, 189-196.

Allemand, D., De Renzis, G., Ciapa, B., Girard, J. P. and Payan, P. (1984). Characterization of valine transport in sea urchin eggs. *Biochim. Biophys. Acta* **772**, 337-346.

Amachi, S., Kimura, K., Muramatsu, Y., Shinoyama, H. and Fujii, T. (2007). Hydrogen peroxide-dependent uptake of iodine by marine Flavobacteriaceae bacterium strain C-21. *Appl. Environ. Microbiol.* **73**, 7536-7541.

Babior, B. M., Curnutte, J. T. and McMurrich, B. J. (1976). The particulate superoxide-forming system from human neutrophils. Properties of the system and further evidence supporting its participation in the respiratory burst. *J. Clin. Invest.* **58**, 989-996.

Beltrán, C., Zapata, O. and Darszon, A. (1996). Membrane potential regulates sea urchin sperm adenylylcyclase. *Biochemistry* **35**, 7591-7598.

Berking, S., Czech, N., Gerharz, M., Herrmann, K., Hoffmann, U., Raifer, H., Sekul, G., Siefker, B., Sommerei, A. and Vedder, F. (2005). A newly discovered oxidant defence system and its involvement in the development of *Aurelia aurita* (Scyphozoa, Cnidaria): reactive oxygen species and elemental iodine control medusa formation. *Int. J. Dev. Biol.* **49**, 969-976.

Blanton, M. L. and Specker, J. L. (2007). The hypothalamic-pituitary-thyroid (HPT) axis in fish and its role in fish development and reproduction. *Crit. Rev. Toxicol.* **37**, 97-115.

Bus, J. S. and Gibson, J. E. (1984). Paraquat: model for oxidant-initiated toxicity. *Environ. Health Perspect.* **55**, 37-46.

Campbell, R. K., Satoh, N. and Degnan, B. M. (2004). Piecing together evolution of the vertebrate endocrine system. *Trends Genet.* **20**, 359-366.

Campos, M. (1997). New approach to evaluating dissolved iodine speciation in natural waters using cathodic stripping voltammetry and a storage study for preserving iodine species. *Mar. Chem.* **57**, 107-117.

Carrasco, N. (1993). Iodide transport in the thyroid gland. *Biochim. Biophys. Acta* **1154**, 65-82.

Chang, M. H., Lin, H. C. and Hwang, P. P. (1997). Effects of cadmium on the kinetics of calcium uptake in developing tilapia larvae, *Oreochromis mossambicus*. *Fish Physiol. Biochem.* **16**, 459-470.

Chino, Y., Saito, M., Yamasu, K., Suyemitsu, T. and Ishihara, K. (1994). Formation of the adult rudiment of sea urchins is influenced by thyroid hormones. *Dev. Biol.* **161**, 1-11.

Cross, A. R. and Jones, O. T. G. (1986). The effect of the inhibitor diphenylene iodonium on the superoxide-generating system of neutrophils. Specific labelling of a component polypeptide of the oxidase. *Biochem. J.* **237**, 111-116.

Davidson, B., Soodak, M., Strout, H. V., Neary, J. T., Nakamura, C. and Maloof, F. (1979). Thiourea and cyanamide as inhibitors of thyroid peroxidase: the role of iodide. *Endocrinology* **104**, 919-924.

Davis, J. P., Keenan, C. L. and Stephens, G. C. (1985). Na^+-dependent amino-acid transport in bacteria-free sea-urchin larvae. *J. Comp. Physiol. B* **156**, 121-127.

Dohán, O. and Carrasco, N. (2003). Advances in Na^+/I^- symporter (NIS) research in the thyroid and beyond. *Mol. Cell. Endocrinol.* **213**, 59-70.

Eales, J. G. (1997). Iodine metabolism and thyroid-related functions in organisms lacking thyroid follicles: are thyroid hormones also vitamins? *Proc. Soc. Exp. Biol. Med.* **214**, 302-317.

Emlet, R. B. (1986). Facultative planktotrophy in the tropical echinoid *Clypeaster rosaceus* (Linnaeus) and a comparison with obligate planktotrophy in *Clypeaster subdepressus* (Gray) (Clypeasteroida, Echinoidea). *J. Exp. Mar. Biol. Ecol.* **95**, 183-202.

Eskandari, S., Loo, D. D., Dai, G., Levy, O., Wright, E. M. and Carrasco, N. (1997). Thyroid Na^+/I^- symporter. Mechanism, stoichiometry, and specificity. *J. Biol. Chem.* **272**, 27230-27238.

Foerder, C. A., Klebanoff, S. J. and Shapiro, B. M. (1978). Hydrogen peroxide production, chemiluminescence, and the respiratory burst of fertilization: interrelated events in early sea urchin development. *Proc. Natl. Acad. Sci. USA* **75**, 3183-3187.

Fuge, R. and Johnson, C. C. (1986). The geochemistry of iodine – a review. *Environ. Geochem. Health* **8**, 31-54.

Gensemer, R. W., DeForest, D. K., Stenhouse, A. J., Higgins, C. J. and Cardwell, R. D. (2006). Aquatic toxicity of cyanide. In *Cyanide in Water and Soil: Chemistry, Risk and Management* (ed. D. A. Dzombak, R. S. Ghosh and G. M. Wong-Chong), pp. 251-284. Boca Raton, FL: Taylor and Francis Group.

Gouy, M., Guindon, S. and Gascuel, O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* **27**, 221-224.

Hamre, K., Opstad, I., Espe, M., Solbakken, J., Hemre, G. I. and Pittman, K. (2002). Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural zooplankton or Artemia. *Aquacult. Nutr.* **8**, 139-148.

Herring, J. R. and Liss, P. S. (1974). New method for determination of iodine species in seawater. *Deep Sea Res.* **21**, 777-783.

Heyland, A. (2004). *Thyroid Hormone-Like Function in Echinoids: A Modular Signaling System Coopted for Larval Development and Critical for Life History Evolution*. Gainesville, FL: University of Florida.

Heyland, A. and Hodin, J. (2004). Heterochronic developmental shift caused by thyroid hormone in larval sand dollars and its implications for phenotypic plasticity and the evolution of nonfeeding development. *Evolution* **58**, 524-538.

Heyland, A. and Moroz, L. L. (2005). Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. *J. Exp. Biol.* **208**, 4355-4361.

Heyland, A. and Moroz, L. L. (2006). Signaling mechanisms underlying metamorphic transitions in animals. *Integr. Comp. Biol.* **46**, 743-759.

Heyland, A., Bodnarova, M. and Moroz, L. L. (2004). Thyroid hormone like metabolism and function in complex life histories. *Integr. Comp. Biol.* **44**, 568.

Heyland, A., Hodin, J. and Reitzel, A. M. (2005). Hormone signaling in evolution and development: a non-model system approach. *Bioessays* **27**, 64-75.

Heyland, A., Price, D. A., Bodnarova-Buganova, M. and Moroz, L. L. (2006). Thyroid hormone metabolism and peroxidase function in two non-chordate animals. *J. Exp. Zool. B Mol. Dev. Evol.* **306**, 551-566.

Heyland, A. Reitzel, A. M. and Degnan, S. (2011). Emerging patterns in the regulation of marine invertebrate settlement and metmorphosis. In *Mechanisms of Life History Evolution* (ed. T. H. Flatt and A. Heyland), pp. 29-42. Oxford: Oxford University Press.

Hunn, J. B. and Fromm, P. O. (1966). In vivo uptake of radioiodide by rainbow trout. *J. Water Pollut. Control Fed.* **38**, 1981-1985.

Johnson, L. G. and Cartwright, C. M. (1996). Thyroxine-accelerated larval development in the crown-of-thorns starfish, *Acanthaster planci*. *Biol. Bull.* **190**, 299-301.

Jung, H. (2002). The sodium/substrate symporter family: structural and functional features. *FEBS Lett.* **529**, 73-77.

Kanai, Y., Lee, W. S., You, G. F., Brown, D. and Hediger, M. A. (1994). The human kidney low affinity Na^+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose. *J. Clin. Invest.* **93**, 397-404.

Klebanoff, S. J., Foerder, C. A., Eddy, E. M. and Shapiro, B. M. (1979). Metabolic similarities between fertilization and phagocytosis. Conservation of a peroxidatic mechanism. *J. Exp. Med.* **149**, 938-953.

Küpper, F. C., Schweigert, N., Gall, E. A., Legendre, J. M., Vilter, H. and Kloareg, B. (1998). Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. *Planta* **207**, 163-171.

Küpper, F. C., Kloareg, B., Guern, J. and Potin, P. (2001). Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. *Plant Physiol.* **125**, 278-291.

Küpper, F. C., Müller, D. G., Peters, A. F., Kloareg, B. and Potin, P. (2002). Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of sporophytes of laminariales. *J. Chem. Ecol.* **28**, 2057-2081.

Küpper, F. C., Gaquerel, E., Boneberg, E.-M., Morath, S., Salaün, J.-P. and Potin, P. (2006). Early events in the perception of lipopolysaccharides in the brown alga *Laminaria digitata* include an oxidative burst and activation of fatty acid oxidation cascades. *J. Exp. Bot.* **57**, 1991-1999.

Küpper, F. C., Carpenter, L. J., McFiggans, G. B., Palmer, C. J., Waite, T. J., Boneberg, E. M., Woitsch, S., Weiller, M., Abela, R., Grolimund, D. et al. (2008). Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. *Proc. Natl. Acad. Sci. USA* **105**, 6954-6958.

Lacroix, L., Pourcher, T., Magnon, C., Bellon, N., Talbot, M., Intaraphairot, T., Caillou, B., Schlumberger, M. and Bidart, J. M. (2004). Expression of the apical iodide transporter in human thyroid tissues: a comparison study with other iodide transporters. *J. Clin. Endocrinol. Metab.* **89**, 1423-1428.

Luther, G. W., Wu, J. F. and Cullen, J. B. (1995). Redox chemistry of iodine in seawater – frontier molecular-orbital theory considerations. *Adv. Chem. Ser.* **244**, 135-155.

Miller, A. E. M. and Heyland, A. (2010). Endocrine interactions between plants and animals: implications of exogenous hormone sources for the evolution of hormone signaling. *Gen. Comp. Endocrinol.* **166**, 455-461.

Moren, M., Opstad, I., Van der Meeren, T. and Hamre, K. (2006). Iodine enrichment of *Artemia* and enhanced levels of iodine in Atlantic halibut larvae (*Hippoglossus hippoglossus* L.) fed the enriched *Artemia*. *Aquacult. Nutr.* **12**, 97-102.

Moren, M., Sloth, J. J. and Hamre, K. (2008). Uptake of iodide from water in Atlantic halibut larvae (*Hippoglossus hippoglossus* L.). *Aquaculture* **285**, 174-178.

Obinger, C., Regelsberger, G., Strasser, G., Burner, U. and Peschek, G. A. (1997). Purification and characterization of a homodimeric catalase-peroxidase from the cyanobacterium *Anacystis nidulans*. *Biochem. Biophys. Res. Commun.* **235**, 545-552.

Paris, M., Escriva, H., Schubert, M., Brunet, F., Brtko, J., Ciesielski, F., Roecklin, D., Vivat-Hannah, V., Jamin, E. L., Cravedi, J. P. et al. (2008). Amphioxus postembryonic development reveals the homology of chordate metamorphosis. *Curr. Biol.* **18**, 825-830.

Rodriguez, A. M., Perron, B., Lacroix, L., Caillou, B., Leblanc, G., Schlumberger, M., Bidart, J. M. and Pourcher, T. (2002). Identification and characterization of a putative human iodide transporter located at the apical membrane of thyrocytes. *J. Clin. Endocrinol. Metab.* **87**, 3500-3503.

Saito, M., Seki, M., Amemiya, S., Yamasu, K., Suyemitsu, T. and Ishihara, K. (1998). Induction of metamorphosis in the sand dollar *Peronella japonica* by thyroid hormones. *Dev. Growth Differ.* **40**, 307-312.

Shrivastava, A., Tiwari, M., Sinha, R. A., Kumar, A., Balapure, A. K., Bajpai, V. K., Sharma, R., Mitra, K., Tandon, A. and Godbole, M. M. (2006). Molecular iodine induces caspase-independent apoptosis in human breast carcinoma cells involving the mitochondria-mediated pathway. *J. Biol. Chem.* **281**, 19762-19771.

Silverstone, M., Galton, V. A. and Ingbar, S. H. (1978). Observations concerning the metabolism of iodine by polyps of *Aurelia aurita*. *Gen. Comp. Endocrinol.* **34**, 132-140.

Smanik, P., Liu, Q., Furminger, T., Ryu, K., Xing, S., Mazzaferri, E. and Jhiang, S. (1996). Cloning of the human sodium iodide symporter. *Biochem. Biophys. Res. Commun.* **226**, 339-345.

Smyth, P. P. A. (2003). Role of iodine in antioxidant defence in thyroid and breast disease. *Biofactors* **19**, 121-130.

Sodergren, E., Weinstock, G. M., Davidson, E. H., Cameron, R. A., Gibbs, R. A., Angerer, R. C., Angerer, L. M., Arnone, M. I., Burgess, D. R., Burke, R. D. et al. (2006). The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science* **314**, 941-952.

Spangenberg, D. B. (1974). Thyroxine in early strobilation in *Aurelia aurita*. *Am. Zool.* **14**, 825-831.
Truesdale, V. W. (1978). Iodine in in-shore and off-shore marine waters. *Mar. Chem.* **6**, 1-13.
Truesdale, V. W. (1993). The chemical-reduction of molecular-iodine added to seawater, modeled as a system of linked 1st order reaction. *Mar. Chem.* **42**, 147-166.
Tsunogai, S. (1971). Iodine in deep water of ocean. *Deep Sea Res.* **18**, 913-919.
Venturi, S. and Venturi, M. (2009). Iodine, thymus, and immunity. *Nutrition* **25**, 977-979.
Wong, G. T. F. (1991). The marine geochemistry of iodine. *Rev. Aquat. Sci.* **4**, 45-73.
Wong, J. L. and Wessel, G. M. (2005). Reactive oxygen species and Udx1 during early sea urchin development. *Dev. Biol.* **288**, 317-333.
Wong, J. L., Créton, R. and Wessel, G. M. (2004). The oxidative burst at fertilization is dependent upon activation of the dual oxidase Udx1. *Dev. Cell* **7**, 801-814.
Wood, R. L. and Legg, P. G. (1970). Peroxidase activity in rat liver microbodies after amino-triazole inhibition. *J. Cell Biol.* **45**, 576-585.
Wright, S. H. and Manahan, D. T. (1989). Integumental nutrient uptake by aquatic organisms. *Annu. Rev. Physiol.* **51**, 585-600.
Wright, E. M. and Turk, E. (2004). The sodium/glucose cotransport family SLC5. *Pflügers Arch.* **447**, 510-518.
Zoeller, R. T., Tan, S. W. and Tyl, R. W. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis. *Crit. Rev. Toxicol.* **37**, 11-53.