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Short communication

Growth and survival of California sea cucumbers (*Parastichopus californicus*) cultivated with sablefish (*Anoplopoma fimbria*) at an integrated multi-trophic aquaculture site

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ABSTRACT

In a 12-month field trial we examined the growth and survival of California sea cucumbers (*Parastichopus californicus*) in suspended culture underneath net pens of sablefish (*Anoplopoma fimbria*) at an experimental integrated multi-trophic aquaculture (IMTA) site. We tested the effects of sea cucumber size (small: 7–99 g and large: 100–565 g whole wet weight) and stocking density (12, 17, and 21 ind m^{-2}) on growth and survival in a completely-crossed experimental design. We also compared growth and survival of experimental animals cultured directly under the fish pens with control sea cucumbers grown ~250 m away from the farm. The ability of the sea cucumbers to reduce total organic carbon and total nitrogen from the sablefish faeces was also examined. Small experimental animals grew significantly faster than large experimental individuals, the former increasing in whole wet weight by 27–56% over the 12 months and the latter decreasing by 10–33% over the same period. It was concluded that stocking densities of large animals were too high to produce net positive growth. Stocking density had a significant effect on growth of both size classes, lower densities producing higher growth rates, or less negative growth rates in the case of large animals. Small sea cucumbers suspended directly below the sablefish net pens grew significantly faster than control individuals grown ~250 m away from the farm, which had negative growth over the 12-month period. The small sea cucumbers cultured under the net pens had a high survival rate (mean: 99.5%) and their feeding reduced the total organic carbon and total nitrogen contents of the sablefish faeces by an average of 60.3% and 62.3%, respectively, demonstrating their potential as an important organic-reducing component in IMTA. Suspending sea cucumbers below fish net pens, as opposed to growing them on the seabed, makes their collection and monitoring easier and moves them away from potential seabed predators such as sea stars. This study demonstrated that *P. californicus* is well suited to utilise the heavy fraction of waste from a sablefish farm while providing an additional valuable harvestable product.

1. Introduction

There are growing concerns regarding the ability of the environment to sustain the expansion of intensive marine-finfish aquaculture and the effects of inorganic and organic wastes produced at fish farms (Brooks and Mahnken, 2003; Folke and Kautsky, 1989; Mayor and Solan, 2011; Pillay, 2004; Wu, 1995). Integrated multi-trophic aquaculture (IMTA) offers a natural means to utilise some of the waste material from finfish aquaculture as inputs into the production of lower-trophic-level crops of commercial value, increasing both the environmental and economical sustainability of the aquaculture operation by reducing waste output and increasing product diversity (Troell et al., 2009). A successful fully-integrated IMTA system closely mimics natural ecosystem function (Folke and Kautsky, 1992), utilising species at several trophic levels to consume different types of waste: dissolved nutrient fractions can be absorbed by macroalgae, fine particulates consumed by filter-feeding shellfish, and heavier particulates taken up by deposit feeders (Chopin et al., 2001).

Commercial-scale IMTA is established on the Atlantic coast of Canada, where blue mussels (*Mytilus edulis*) and kelp (*Saccharina latissima* and *Alaria esculenta*) are grown adjacent to Atlantic salmon (*Salmo salar*) (Neori et al., 2007; Reid et al., 2009; Ridler et al., 2007). On the Pacific coast of Canada, the pre-commercial-scale testing of IMTA with sablefish (*Anoplopoma fimbria*) in co-culture with Pacific scallops [an unconfirmed hybrid between the Japanese scallop (*Mizuhopecten yessoensis*) and the weathervane scallop (*Patinopecten caurinus*)], kelp (*S. latissima*), and sea cucumbers (*Parastichopus californicus*) has recently begun at a site

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in Kyuquot Sound on the northwest coast of Vancouver Island, British Columbia (BC), Canada. An important part of these early IMTA endeavours on the Pacific coast is the initial testing of potentially suitable invertebrate species. The present study examined growth and survival of deposit-feeding California sea cucumbers (*P. californicus*) consuming heavier particulate fractions of sablefish waste.

Deposit-feeding sea cucumbers can recycle nutrients and bioturbate sediments (Uthicke, 1999), potentially limiting anaerobic bacterial growth and the formation of anoxic zones in highly enriched benthic environments, as may occur underneath shellfish and finfish farms. The majority of studies that have examined co-culture of sea cucumbers with other organisms have utilised shellfish species. In New Zealand, the Australasian sea cucumber (*Australostichopus mollis*) had high survival and growth rates when cultured below green-lipped mussels (*Perna canaliculus*) (Slater and Carton, 2007). In China, sea cucumbers (*Stichopus japonicus*) grew well when co-cultured with scallops (*Chlamys farreri* and *Argopecten irradians irradians*) and oysters (*Crassostrea gigas*) in both closed and open systems (Zhou et al., 2006). In BC, California sea cucumbers (*P. californicus*) suspended below Pacific oysters (*C. gigas*) grew well and had high survival rates (Paltzat et al., 2008), and in China, shrimp are commonly raised successfully in co-culture with sea cucumbers (Martinez-Porchas et al., 2010; Yaqing et al., 2004). Few studies, however, have utilised sea cucumbers to consume waste material at finfish farms. A recent study on juvenile sea cucumbers (*Apostichopus japonicus*) cultured below an open net pen containing red sea bream (*Pagrus major*), found high survivorship and significantly higher growth rate than in control individuals held away from the farm (Yokoyama, 2013).

The California sea cucumber, found from the Gulf of Alaska to Baja California (Brumbaugh, 1980), is a deposit feeder which collects organic matter with peltate feeding tentacles to which detritus adheres and is drawn into the mouth (Cameron and Fankboner, 1984). Feeding activity slows in October to November before visceral atrophy during aestivation in the winter. This process involves reabsorbing of the gut, gonad, respiratory trees, and circulatory system, resulting in an approximate 25% loss of body weight (Fankboner and Cameron, 1985). *P. californicus* is capable of consuming salmon net-pen fouling material, resulting in increased muscle development (Ahlgren, 1998), and has been shown to grow and survive well when suspended below cultured Pacific oysters (Paltzat et al., 2008). Results from both of these studies indicate that the California sea cucumber has potential as an organic-extractive species in IMTA. Sea cucumbers can also fetch a high market price, with a retail value in China of over US $400 kg^{-1} for some species in 2004 (Chen, 2004; Hamel et al., 2001), and thus can contribute to the profitability of a fish farm as a secondary cash crop. There is already a proven Chinese market for *P. californicus*, with a rather lucrative wild fishery occurring in BC [landed value of CAN $3.36 million in 2011 (Fisheries and Oceans Canada, online commercial fisheries statistics)].

In the present study, we examined the effect of stocking density on growth and survival of small and large *P. californicus* suspended below, and consuming organic waste from, a sablefish farm in Kyuquot Sound, BC. Growth and survival of experimental sea cucumbers were compared with control animals cultured away from the sablefish farm. The ability of the sea cucumbers to reduce total organic carbon and total nitrogen from the sablefish faeces was also examined.

2. Methods

2.1. Description of site

The aquaculture site was located in Kyuquot Sound on the west coast of Vancouver Island, BC (50° 02′ 47.7″ N, 127° 17′ 48.6″ W) and comprised a single array of 12 anchored fish cages, each 15 × 15 × 20 m (L × W × H), in water ~30 m deep. At the beginning of the experiment, two cages were populated with 25,000 sablefish (*A. fimbria*) each. Fish biomass was maintained in each cage over the experimental duration with stocking density being reduced through size grading; by the end of the experiment there were five stocked cages. Fish size increased from ~500 g at the start of the experiment to ~1500 g at its completion. Fish were fed daily at approximately 5% of their body weight with "black cod" feed produced by Taplow Feeds (Vancouver, BC). Ingredients and concentration of various dietary components in the feed are given in Table 1. Also on site during the experiment was a 15-m wide array of Pacific scallops suspended in lantern nets which were 1 m apart, at 7 to 12 m depth, and located downstream and parallel to the fish cages. Downstream from the shellfish there was a grid of 20 lines of kelp (*S. latissima*), each line being 50 m in length with adjacent parallel lines spaced 1 m apart. Tidal circulation at the farm system drives a residual flow that moves water primarily in a direction across the system components, i.e. through the fish cages and then downstream to the suspended shellfish and then farther downstream to the kelp grid.

2.2. Collection of animals

Large (whole wet weight: 100–565 g) sea cucumbers were collected by scuba divers from a natural population in Cachalot Inlet, Kyuquot Sound (49° 59′ 54.0″ N, 127° 8′ 23.0″ W) on 13 November 2008. Small (whole wet weight: 7–99 g) sea cucumbers were collected by hand from a population that had settled on an abandoned shellfish farm structure in the same inlet. Due to their low density and cryptic behaviour, collecting sufficient small individuals in a more natural environment was not possible. Animals were placed in containers with ambient seawater, transferred to the IMTA site, placed in cages at approximately 15-m depth, and held for 5 days until experimental setup. A second collection of sea cucumbers was made in the same inlet on 11 March 2009 to furnish additional control cages which were added at this time.

2.3. Experimental setup and sampling

On 17 November 2008 sea cucumbers were transferred from their holding cages to a flow-through storage tank (L × W × H: 3 × 1 × 1 m), supplied with ambient seawater pumped from ~5-m depth, and held for 24 h to allow for acclimatisation and expulsion of gut contents. They were measured for whole wet weight (i.e. the entire wet weight of the intact sea cucumber), separated into small (<100 g) and large (>100 g) size categories, and allocated to experimental or control cages. Standard plastic oyster-culture trays (L × W × H: 57 × 57 × 21 cm, surface area: 1.13 m^2) were modified with mesh (20-mm and 5-mm mesh for large and small size categories, respectively) added under the lids to aid in sea cucumber containment and with

Table 1
Composition of "black cod" (sablefish, *Anoplopoma fimbria*) feed produced by Taplow Feeds, Vancouver, British Columbia, Canada. Sample from September 2007. Information is as listed on fish-feed bags.

Dietary component	Concentration	
Crude protein	46%	
Crude fat	18%	
Crude fibre	2%	Max
Moisture	10%	Max
Ash	10%	Max
Vitamin A	8000 IU kg^{-1}	Min
Vitamin D	1000 IU kg^{-1}	Min
Vitamin E	175 IU kg^{-1}	Min
Calcium	1%	Actual
Sodium	1%	Actual
Phosphorus	0.65%	Actual

Ingredients: Fish meal, organic wheat, fish oil, wheat gluten, calcium propionate.

0.5-mm mesh on the tray bases to retain material settling out of the water column.

There were three stocking densities for both size categories of sea cucumbers: “low”, “medium”, and “high” [14, 19, and 24 ind cage^{-1} or 12.4, 16.8, and 21.2 ind m^{-2}, respectively (note that m^{-2} values include tray bottoms, sides, and tops)]. There were three replicate cages for each size/density combination, i.e. six treatments and 18 cages in total. Experimental densities were selected based on sea cucumber availability and findings from laboratory feeding experiments (L. Hannah, pers. obs).

The 18 experimental cages were randomly distributed between two specially-designed metal frames (L × W: 15 × 1 m) which extended the length of a fish net pen. Cages were attached to the frames and then each was lowered and secured in place, suspended ~3 m directly below the bottom of the fish net pens. For both size categories, three control cages were stocked with a medium density of sea cucumbers (19 ind cage^{-1} or 16.8 ind m^{-2}) and lowered to the seabed at ~25 m depth. These controls were placed ~250 m upstream from the experimental set up. The separation of the controls from the fish farm was such that waste material from the fish pens was unlikely to impinge on them, both experimental and control cages presumably being exposed to comparable water quality, oceanographic conditions, and natural seston levels. At a later date (24 March 2009), a further six control cages were added at the same control location, three containing a medium density of small sea cucumbers (19 ind cage^{-1} or 16.8 ind m^{-2}) and three containing a medium density of large individuals (17 ind cage^{-1} or 15.0 ind m^{-2}, the lower large size-class density being due to limited numbers of animals available at the time). These cages differed from the original controls in that they were suspended off the seafloor at ~15 m depth. These additional control cages were added as a result of unexpected high mortality rates, due to predation, in the original seabed control cages. Additional sea cucumbers (40–50 individuals) were suspended at 10 m depth in trays ~100 m away from the sablefish cages. Five to ten of these animals were randomly chosen at each sampling interval and dissected to assess the condition of the alimentary canal and to determine when the period of aestivation occurred.

Experimental and control cages were sampled approximately every 2 months (i.e. 18 November 2008, 28 January, 26 March, 19 May, 29 July, 23 September, and 2 December 2009). Sea cucumbers were retrieved from trays, put into labelled mesh bags, and held for 24 h in a flow-through storage tank (L × W × H: 3 × 1 × 1 m) supplied with ambient seawater pumped from ~5 m depth. After 24-h acclimatisation, sea cucumbers were measured for whole wet weight and then returned to the labelled bags and held in the storage tank until being replaced in the experimental and control cages later in the day.

Growth rates were calculated as: (1) percentage change in mean whole wet weight from the beginning of the experiment, using an average of the total number of sea cucumbers per tray, (2) absolute growth rate (AGR, in g d^{-1}), and (3) specific growth rate (SGR, in % change d^{-1}) (An et al., 2007; Battaglene et al., 1999) using the following formulae:

(1) $\% \text{ change} = ((W_2 - W_1) / W_1) \times 100$
(2) $\text{AGR} = (W_2 - W_1) / D$
(3) $\text{SGR} = (\log_e W_2 - \log_e W_1) / D \times 100$

where W_2 is the mean whole wet weight (g) of sea cucumbers in a cage at the time of sampling, W_1 is the mean whole wet weight (g) of sea cucumbers in the same cage at the beginning of the experiment, and D is the number of days between the two sample dates.

At the end of the experimental period in December 2009, samples of sablefish commercial feed, sablefish faeces, and sea cucumber faeces (from both experimental and control trays) were collected for determination of total organic carbon (TOC), total nitrogen (TN), and organic matter contents. These samples were stored at −80 °C until analysed (described in Section 2.4). Temperature data loggers (HOBO® Pro v2, Onset Computer Corporation, Pocasset, MA, USA) attached to two experimental trays, one suspended control tray, and one seabed control tray recorded temperatures every 2 h throughout the experiment. In addition, temperature and salinity were measured at each sampling period at both experimental and control locations using a handheld YSI salinity/temperature probe (YSI Inc., Yellow Springs, OH, USA) in water collected from 0, 1, 5, 10, and 23-m depths using a delayed-release water sampler. Secchi-disk depth was recorded away from the direct influence of particulates originating from the fish pens at each sampling period to assess general background turbidity levels.

2.4. Analysis of sediment samples

Samples were freeze-dried and ground to a uniform consistency with an acid-washed mortar and pestle. Ground samples were stored frozen (−80 °C) in ashed scintillation vials until analysis of TOC and TN, using methods described in Paltzat et al. (2008). TOC and TN data were converted to percentages of total dry weight of sample.

2.5. Data analysis

Percent weight-change data (i.e. change from the beginning of the study) were used to allow the comparison of the two size groups in a two-way repeated measures ANOVA with size (small, large) and stocking density (14, 19, 24 ind cage^{-1}) as grouping factors. Untransformed data were assessed for normality using Shapiro–Wilk's test and for homogeneity of variances with Levene's test and were both normal and homogeneous ($P > 0.05$). After size was determined to be a significant factor ($F_{1,10} = 105.85$, $P < 0.001$), small and large size-class data sets were separated and analysed individually using raw (i.e. not percent change) natural-log transformed results to provide more data points and allow a clearer analysis than by using mean percent-change values. Residuals of natural-log transformed data were assessed for normality and homogeneity of variances with the two tests mentioned above. Both small and large size-class data sets were homogeneous with respect to variances, but were slightly non-normal. Since ANOVA is fairly robust to small departures from normality, we chose to analyse the data with ANOVA. Small and large size-class data sets were each analysed using a one-way repeated measures ANOVA with stocking density (14, 19, 24 ind cage^{-1}) as a grouping factor, followed by Tukey's multiple comparison post-hoc tests to examine significant density and time effects. One-way repeated measures ANOVAs were used to compare seabed controls to medium density experimental treatments, also using natural-log transformed data. These data were assessed for normality and homogeneity using the tests described above. Both small and large size-class data sets were homogeneous with respect to variances, but were slightly non-normal. Again, we chose to use ANOVA for the data analysis as it is fairly robust to small departures from normality. These ANOVAs were followed up with Tukey's multiple comparison post-hoc tests to examine the effects of time within both treatments and the effects of treatment within time, as there was a significant interaction between treatment and time in both small and large size-class datasets. All statistical tests were carried out using SYSTAT® Version 11 (SYSTAT Software Inc., Richmond, CA, USA).

3. Results

3.1. Water temperature, salinity, and turbidity

Temperatures at cage level at the experimental and seabed control sites were very similar (Fig. 1A), reaching an annual low of ~7 °C in February 2009 and peaking in mid-August at ~14 °C. Depth profiles of water temperature (Fig. 1B) and salinity (Fig. 1C) show low temperatures in the low-saline surface water in December 2009, indicating a significant influx of cold freshwater during the winter months

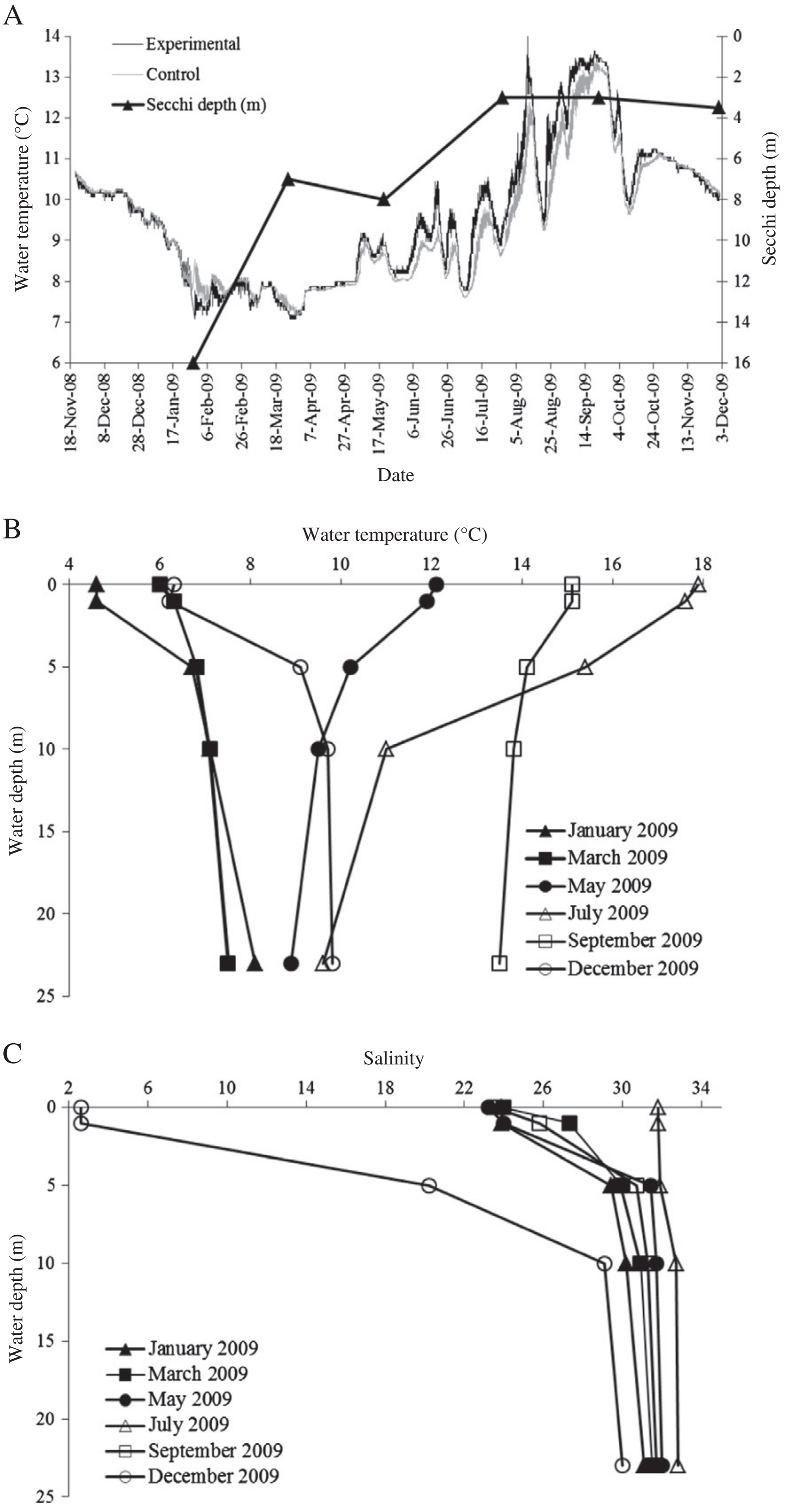

Fig. 1. (A) Water temperatures at seabed control and experimental cages at 23-m depth recorded by data loggers which recorded temperatures every 2 h throughout the experiment. Daily means are plotted. On the secondary Y axis is Secchi disk depth. Depth profiles at each sampling interval for (B) temperature and (C) salinity at the experimental site from water samples collected at 0, 1, 5, 10, and 23-m depths using a delayed-release water sampler.

which pushes down the halocline which is present for the rest of the year. The experimental and control cages were located below this halocline and so experienced low salinity variations (salinities of 29.3–32.8). Temperature and salinity profiles recorded at the control location closely followed the patterns observed at the experimental site and are not shown.

Maximum Secchi depth was observed in late January 2009 (16 m), after which it decreased to 7 m by mid-March 2009 and to 2.5 m in late July 2009. After that, Secchi depth increased to 3.5 m by early December (Fig. 1A).

3.2. Effect of size on sea cucumber growth

Growth rate of small sea cucumbers was significantly higher than that of large individuals when percent change data were analysed (two-way repeated measures ANOVA: $F_{1,10} = 105.85, P < 0.001$). Small sea cucumbers grown under sablefish pens exhibited net positive growth at all densities, increasing in whole wet weight by 26.7–55.9% over the experimental period (Fig. 2A). Large individuals grown under the fish pens had overall negative growth, however, decreasing in whole wet weight by 9.6–32.6% over the same duration (Fig. 2B). Seabed controls for small and large sea cucumbers exhibited overall negative growth during the experiment, the former decreasing by 36.8–47.9% and the latter by 10.9–33.6%.

3.3. Effect of density on sea cucumber growth

Sea cucumber whole wet weight varied significantly with the density of small sea cucumbers, but not with the density of large ones (but only marginally non-significant at $P = 0.057$) (Table 2). In both data sets, the effect of date was significant, but the interaction between date and density was not (Table 2). When data were combined from all sampling

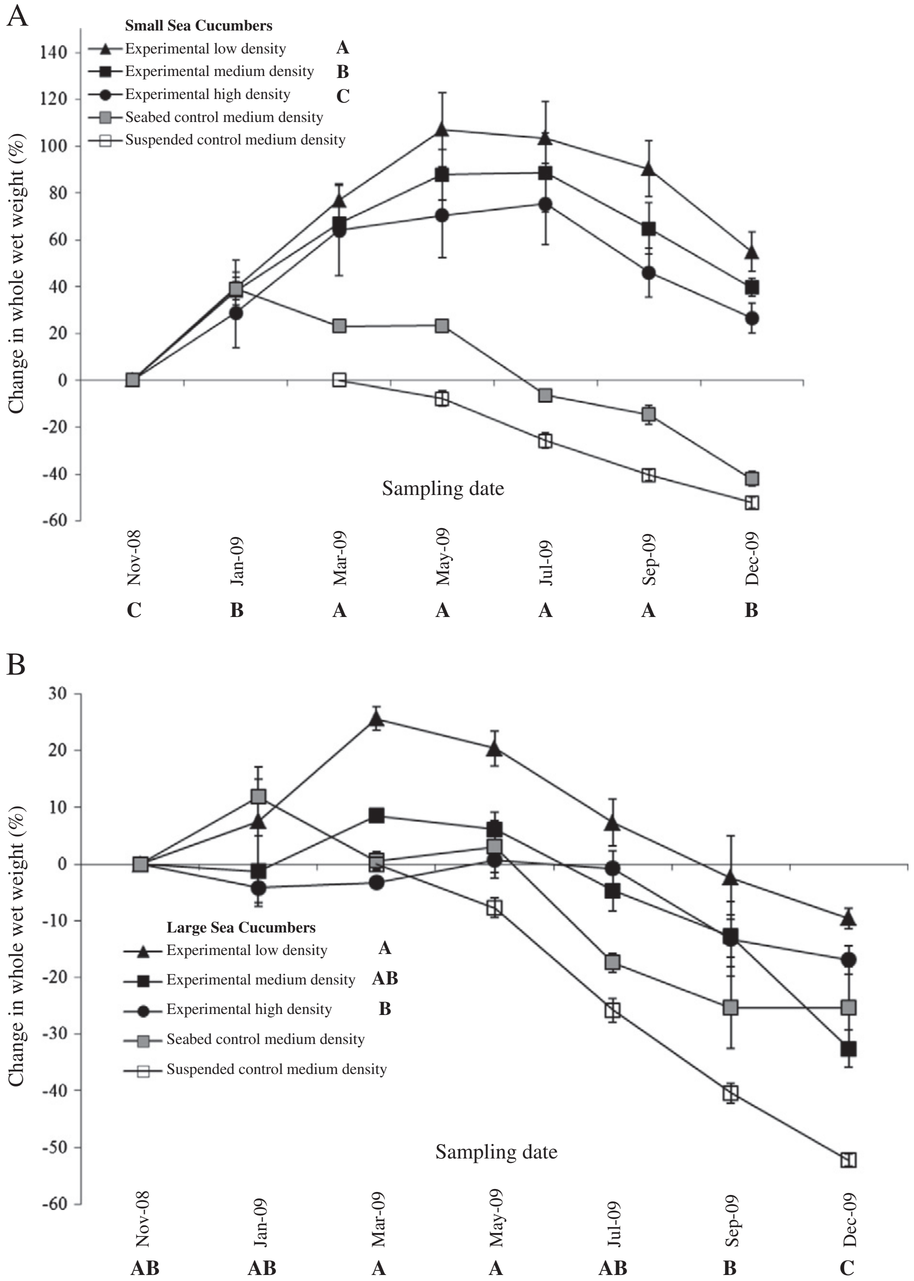

Fig. 2. Mean ($\pm$SE, $n = 3$) percent change in whole wet weight of (A) small (7–99 g whole wet weight in November 2008) and (B) large (100–565 g whole wet weight in November 2008) experimental sea cucumbers (*Parastichopus californicus*) suspended under a sablefish (*Anoplopoma fimbria*) net pen at three stocking densities: 12, 17, and 21 ind m^{-2} (low, medium, and high densities, respectively). Data are also shown for control animals in cages kept on the seabed (17 ind m^{-2}) and in suspended cages (small sea cucumbers: 17 ind m^{-2}, large sea cucumbers: 15 ind m^{-2}) ~250 m from the fish pens. Bold letters underneath dates and next to legends indicate results of Tukey's tests, where dates (across all densities) and densities (across all dates) with different letters differ significantly ($P < 0.05$) from one another.

dates, small sea cucumbers in the low density treatment were significantly heavier than those in both the medium and high density treatments while small individuals held at medium density were significantly heavier than those cultured at high density (Fig. 2A). For the large size-class data set, if $P = 0.057$ is considered significant and followed with a post-hoc multiple comparisons test, then sea cucumbers at low density were significantly heavier than those at high density, there being no other significant pair-wise comparisons (Fig. 2B). Absolute and standard growth rates, which describe daily growth averaged over the experimental duration, also showed a clear decrease from low to medium to high density during the experiment for small sea cucumbers. For large individuals, growth was negative, with the smallest decrease recorded at low density, followed by high, then medium density. Seabed controls also had negative growth (Table 3).

When comparing the medium density seabed control with the medium density experimental treatment, the ANOVA results revealed that treatment, date, and the interaction between the two factors all significantly affected small sea cucumber wet weights (Table 4). Weight of large individuals was significantly affected by date and the interaction between date and treatment but not by treatment alone (Table 4). Experimental small sea cucumbers were significantly heavier than seabed control animals from March until the end of the experiment (Fig. 2A). Medium-density experimental large individuals, however, were only significantly heavier than seabed control animals during July and September (Fig. 2B).

3.4. Effect of sampling date on sea cucumber growth

Wet weights of small sea cucumbers peaked in May in animals at low density (mean ± SE: 114 ± 11.5 g), in July in those at medium and high densities (mean ± SE: 101 ± 9.8 g and 92 ± 7.6 g, respectively), and in January in those in the seabed control (mean ± SE: 73 ± 4.5 g). For large individuals, mean wet weights peaked in March in animals at low and medium densities (mean ± SE: 236 ± 22.8 g and 224 ± 22.9 g, respectively), in May in those at high density (mean ± SE: 199 ± 16.8 g), and in January in those in the seabed control (mean ± SE: 219 ± 9.9 g).

Sea cucumber whole wet weight varied significantly with date for both small and large sea cucumbers, with no significant interaction between date and density (Table 2). When data were combined for all densities and compared by date, weights of small individuals at the experimental onset were significantly lower than in all following months (Fig. 2A). Final weight in December of small animals was significantly lower than that recorded in March, May, July, and September (Fig. 2A). Final weight of large sea cucumbers was significantly lower than in all other months sampled while weights in September were significantly lower than those in March or May, with no other significant pair-wise comparisons (Fig. 2B). The wet weights of both size classes in the seabed control treatment decreased significantly from the onset of the experiment to the end (small individuals: one-way ANOVA, $F_{6,371} = 18.90$, $P < 0.001$; large individuals: one-way ANOVA, $F_{6,310} = 10.85$, $P < 0.001$).

Table 2
Results from one-way repeated measures ANOVAs testing the effect of density and date (both fixed factors) on wet weights of small and large experimental sea cucumbers (*Parastichopus californicus*).

Source of variation	Small				Large			
	df	SS	F	P	df	SS	F	P
Between subjects								
Density	2	6.85	10.36	<0.001	2	1.25	2.92	0.057
Error	161	53.21			129	27.68		
Within subjects								
Date	6	49.33	48.33	<0.001	6	13.60	14.25	<0.001
Date × density	12	1.69	0.83	0.620	12	1.78	0.93	0.512
Error	966	164.33			774	123.08		

Table 3
Absolute growth rate (AGR: change in mean individual whole weight in g d^{-1}) and standard growth rate (SGR: change in mean individual whole weight in % d^{-1}) of small and large sea cucumbers (*Parastichopus californicus*) held at different densities and sampled from November 2008 to December 2009 (378 days).

	Small		Large	
	AGR (g d^{-1})	SGR (% d^{-1})	AGR (g d^{-1})	SGR (% d^{-1})
Experimental low density	0.0796	0.1141	−0.0484	−0.0271
Experimental medium density	0.0563	0.0869	−0.1781	−0.1044
Experimental high density	0.0359	0.0601	−0.0880	−0.0488
Seabed control medium density	−0.0576	−0.1438	−0.1635	−0.1002

3.5. Sea cucumber mortality and aestivation

Sea cucumbers missing from cages were assumed to have died. Cumulative mortality over the experimental period of small sea cucumbers in experimental treatments was low (0–4.2%) while those in seabed control cages suffered higher losses (0–26.3%) (Fig. 3A). Mortality of large animals (low density: 14.3–28.6%, medium density: 10.5–15.8%, high density: 20.8–37.5%) was higher than that of small ones in experimental treatments (Fig. 3B). Large animals at high density suffered the highest losses of the experimental density treatments while large size-class seabed controls had the highest losses (47.4% in each replicate) overall of any treatment (Fig. 3B). Many of the large sea cucumbers died in the latter half of the study.

All ten animals examined in November 2009 had no visible digestive system (data not shown). However, sea cucumbers examined in September and December, as well as at all other times of the year, did have digestive systems.

3.6. Organic carbon and nitrogen contents of samples

Total organic carbon decreased from 47.4 ± 0.2% (mean ± SE) in sablefish faeces to 18.8 ± 5.2% and 18.7 ± 5.2% in faeces from experimental small and large sea cucumbers, respectively, leading to a 60.3–60.6% reduction in %TOC (Table 5). In control treatments away from the influence of the fish farm, sea cucumber faeces had a lower organic content (11.9–12.6%). Total nitrogen decreased from 6.4 ± 0.1% in sablefish faeces to 2.4 ± 0.7% and 2.3 ± 0.2% in faeces from experimental small and large sea cucumbers, respectively,

Table 4
Results from one-way repeated measures ANOVAs testing the effect of treatment (experimental versus control) and date (both fixed factors) on wet weights of small and large experimental (medium density only) and control sea cucumbers (*Parastichopus californicus*).

Source of variation	Small				Large			
	df	SS	F	P	df	SS	F	P
Between subjects								
Treatment	1	39.34	107.14	<0.001	1	0.22	0.83	0.366
Error	102	37.50			75	20.31		
Within subjects								
Date	6	24.91	21.83	<0.001	6	13.67	13.58	<0.001
Date × treatment	6	21.08	18.47	<0.001	6	2.11	2.10	0.052
Error	612	116.40			450	75.50		

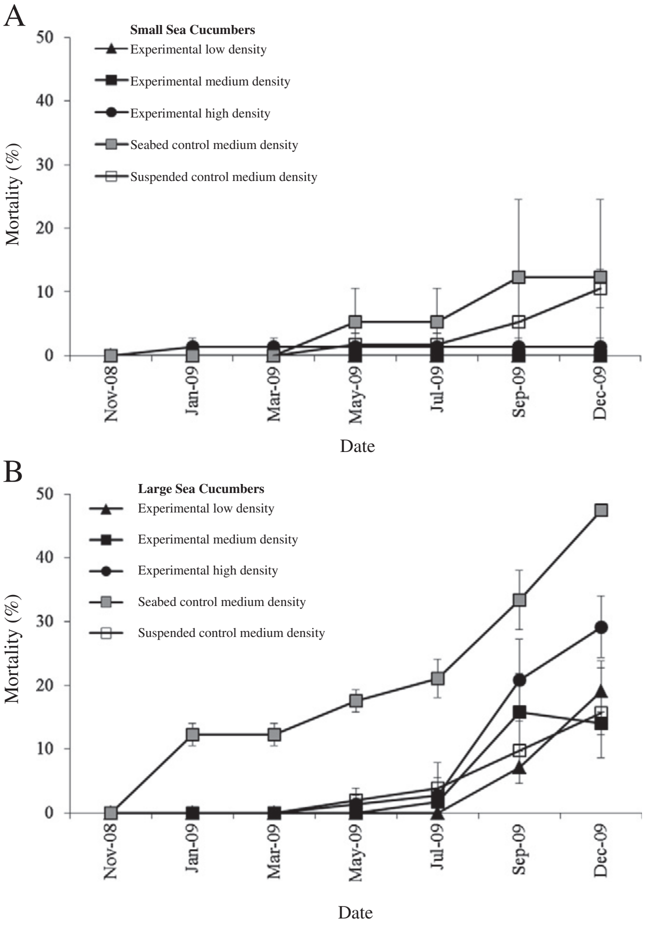

Fig. 3. Mean (±SE, $n = 3$) percent cumulative mortality of (A) small (7–99 g whole wet weight in November 2008) and (B) large (100–565 g whole wet weight in November 2008) experimental sea cucumbers (*Parastichopus californicus*) suspended under a sablefish (*Anoplopoma fimbria*) net pen at three stocking densities: 12, 17, and 21 ind m^{-2} (low, medium, and high densities, respectively). Data are also shown for control animals in cages kept on the seabed (17 ind m^{-2}) and in suspended cages (small sea cucumbers: 17 ind m^{-2}, large sea cucumbers: 15 ind m^{-2}) ~250 m from the fish pens.

leading to a 62.3–64.3% reduction in %TN (Table 5). Values of %TN in control sea cucumber faeces were lower (1.0–1.2%). The C:N ratio increased only slightly from sablefish faeces to experimental sea cucumber faeces (from 7.5 to 7.8–8.2); the C:N ratio was higher in controls (10.5–12.7) (Table 5).

Table 5
Total organic carbon (%TOC) content, total nitrogen (%TN) content, and C/N ratio of sablefish (*Anoplopoma fimbria*) feed, sablefish faeces, and sea cucumber (*Parastichopus californicus*) faeces.

	%TOC			%TN			C/N ratio		
	Mean	SE	*n*	Mean	SE	*n*	Mean	SE	*n*
Sablefish feed	23.49	–	1	2.29	–	1	10.24	–	1
Sablefish faeces & uneaten feed mix	47.37	0.206	2	6.36	0.042	2	7.45	0.082	2
Sea cucumber faeces									
Experimental									
Small	18.79	5.178	2	2.40	0.719	2	7.84	0.208	2
Large	18.68	5.199	2	2.27	0.214	2	8.23	1.530	2
Seabed controls									
Small	12.30	0.290	2	1.17	0.073	2	10.49	0.406	2
Large	11.85	0.368	2	1.03	0.001	2	11.49	0.343	2
Suspended controls									
Small	Lost	–	–	Lost	–	–	Lost	–	–
Large	12.58	–	1	0.99	0.356	2	12.67	–	1

4. Discussion

4.1. Effect of density and size on sea cucumber growth and survival rates

Stocking density is a critical determinant of optimal sea cucumber growth, as shown by increasing growth rate of small sea cucumbers with decreasing stocking density, a pattern also evident with large individuals, albeit with overall negative growth. This stocking-density effect is most certainly related to competition for resources such as food and space. A significant effect of density on growth rate was also found in a recent study of juvenile (initial wet weight: 0.042 to 0.066 g) *A. japonicus* cultured in cages under red sea bream in Japan, where SGR ranged from 2.4% in the highest density treatment (25 ind cage^{-1}) up to 4.1% in the lowest density treatment (1 ind cage^{-1}) (Yokoyama, 2013). Increased growth rate with lower stocking densities was also found in *A. mollis* (mean wet weight: 109 g) grown in trays under a mussel farm in New Zealand for 6 months at a density of 2.5–15 ind m^{-2} (Slater and Carton, 2007).

Decreased growth and increased mortality rates in large compared to small sea cucumbers may have been due to greater competition for food and space with the former individuals. Another factor which may have played a role in the differing growth and mortality rates, however, could have been differential feeding preferences. In *Parastichopus parvimensis*, for example, small individuals prefer fine particulate material on rocks whereas larger ones feed on granular sediments (Yingst, 1982). *P. californicus* may have the same ontogenetic response as a switch in feeding behaviour may be related to habitat shifts — juvenile *Parastichopus californicus* are cryptic and often found feeding in areas where fine particulate matter collects, such as amongst kelp holdfasts and rocky crevices (Shiell, 2004). Further research is required, however, to determine the feeding preferences of varying size classes of *P. californicus*. Size dissimilarities may have also resulted from differences in gamete production or aestivation between the two size groups (see Section 4.2).

The organic content of their food is a factor likely to influence maximal stocking density of sea cucumbers. The mix of fish faeces and uneaten fish feed that the experimental sea cucumbers consumed in the present study was rich in organic matter (%TOC: 47.4%) and appeared to be a suitable food for the sea cucumbers, promoting growth in the small individuals. It is likely that this diet allowed a higher stocking density than would otherwise have been possible, as suggested for the sea cucumber *A. mollis* for which artificial diets rich in organic matter were found to increase nutrient uptake and potentially allow increased stocking densities (Zamora and Jeffs, 2011). The organic content of the food being ingested by sea cucumbers may change over time, however, as they may process food multiple times by ingesting their own faeces. This is supported by a concurrent laboratory study that indicated much higher TOC levels (average 34.6%) in sea cucumber faeces resulting from sablefish faeces having been processed only once by sea cucumbers in a controlled feeding experiment (Hannah, unpublished data).

The biomass of experimental small sea cucumbers held at low, medium, and high densities increased from 692, 920, and 1131 g m^{-2} at experimental onset to 1037, 1225, and 1363 g m^{-2} at the end of the 12-month trial, respectively. Biomass of large individuals at the same three densities decreased from 2330, 3473, and 4193 g m^{-2} at the start to 1697, 2008, and 2460 g m^{-2} at the end, respectively. These values are much higher than the biomass found to be growth-limiting in the sea cucumber *Holothuria scabra* in net pens and hatchery conditions [i.e. 200–250 g m^{-2} (Battaglene et al., 1999; Purcell and Simutoga, 2008)]. Optimal sea cucumber density may not have been tested in the present study and higher growth rates may be achievable at lower stocking densities. The density which optimises sea cucumber growth, survival, nutrient absorption, and the processing of waste material may be different from one optimising growth alone.

4.2. Effect of season on sea cucumber growth and feeding rates

Gamete development in *P. californicus* typically occurs in November through June, followed by spawning in June and July (Cameron, 1980). The annual cycle of aestivation in Kyuquot Sound occurred in October and November during the present experiment, with re-growth likely having taken place in December, similar to populations in the Strait of Georgia, BC (Fankboner and Cameron, 1985). Seasonal changes in sea cucumber physiology due to gamete production and aestivation would likely influence somatic growth by reducing the energy available for somatic production and may have at least partially accounted for the growth differences between small and large sea cucumbers in the present study.

Small sea cucumbers dissected throughout the year generally did not contain gametes and were of a size not likely to be sexually mature. It is probable that the weight decrease in large experimental and control animals between May and July – a decrease not seen in small experimental individuals – was at least partly due to spawning. In addition, the decreases in wet weight in all treatments between September and December were most probably due, at least in part, to aestivation — although there is some evidence that small sea cucumbers of the size used may undergo only limited aestivation (L. Hannah, pers. obs.) so other contributing factors, such as reduced temperatures and feeding rates are likely responsible for the weight decrease in small individuals.

The feeding reduction or cessation characterising this aestivation period has important implications in an IMTA context as it can result in decreased finfish waste consumption during this time. Particulate organic and nitrogen and total phosphorus contents in sea cucumber (*A. japonicus*) culture pond sediments have been reported to increase during aestivation, while they decreased during periods of feeding (Ren et al., 2011). There are a number of potential ways to deal with the problem of feeding cessation and lack of organic stripping during aestivation. Utilising both adult and juvenile sea cucumbers would ensure that some sediment processing continues, since fewer juveniles undergo aestivation (L. Hannah, pers. obs.). Another approach, practised in Japan, is to use different sea cucumber species to reduce the effects of aestivation. In one study, organic loading was reduced by up to 50% in both seasons by using *Holothuria pervicax* in summer and *A. japonicas* in winter as each was adapted to feed in that particular season (S. Kadowaki, Kagoshima University, Japan, pers. comm.). Another potential method would be to utilise other species of deposit feeders that do not aestivate or hibernate (e.g. sea urchins, prawns/shrimps, and deposit-feeding worms). For example, the polychaete *Capitella* sp. markedly enhanced the decomposition of organically-enriched sediments below fish farms (Kinoshita et al., 2008; Wada et al., 2008). Such opportunistic polychaetes are one of a group of burrowers and bioturbators commonly encountered in organically-enriched areas such as fish farms (Brown et al., 1987; Tsutsumi, 1987) and naturally occurring individuals of *Capitella* sp. were, in fact, found feeding on the organic material in cages in the present study (L. Hannah, pers. obs.). Adding another seabed-based deposit feeder to an IMTA system would not only help with the seasonal fluxes in sea cucumber feeding rates, but it could also address the continued, though likely reduced, problem of organic enrichment of the seabed below fish farms by fish waste not intercepted by suspended sea cucumber culture.

4.3. Suspended sea cucumber culture compared to sea ranching

An alternative to suspended culture of sea cucumbers below fish farms is sea ranching, in which juvenile animals are released onto the seabed, often with minimal or no containment structures, to be captured at a later date. This has proven to be a very effective culturing method for sea cucumbers in some areas, with relatively low investment and high returns (Chen, 2004; Gavrilova and Kucheryavenko, 2010; Purcell and Simutoga, 2008). However, sea ranching may not be suitable for fish farms due to potentially unsuitable substrate under the pens (Chen, 2004). Also, sea ranching may result in higher predation, as indicated in the present study where animals on the seabed (even though in cages) suffered much higher mortality rates than those in suspended cages. Furthermore, though requiring initial engineering and structural investment, suspended culture may simplify collection and monitoring of animals.

4.4. Conclusions

This study demonstrated that *P. californicus* is well suited to utilise the heavy fraction of waste from a sablefish farm as a component of an IMTA system. Small sea cucumbers consuming fish waste grew significantly faster than seabed control individuals held away from the site and markedly reduced the total organic carbon and nitrogen of sablefish faeces. Suspending the sea cucumbers below fish net pens makes their collection and monitoring easier than in bottom ranching and moves them away from benthic predators, reducing mortality. Although the growth and survival rates of large sea cucumbers were relatively low, they could potentially be improved by reducing stocking density, which may also increase growth rate in small animals. Adult sea cucumbers experience seasonal fluctuations in feeding and growth (and presumably organic matter reduction) due to gonad development/spawning and aestivation. A mix of sea cucumber size classes would aid in balancing such seasonal influences as well as provide a staggered harvest. Another potential solution to the seasonal effect of sea cucumber feeding is to use a variety of species of deposit feeders (e.g. sea urchins, prawns/shrimps, and deposit-feeding worms). Further optimisation could be achieved through the improvement of the sea cucumber cage design; cages need to protect their occupants from predation and prevent them from escaping while permitting organic matter ingress through the roof and sides of the units but not egress through the floor. Sea cucumbers have the potential to reduce fish-farm waste while providing an additional remunerative product and may be a valuable addition to IMTA systems to ameliorate the problem of the settlement of heavier solids.

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