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Relationship between shell weight and cadmium content in whole digestive gland of the Japanese scallop *Patinopecten yessoensis* (Jay)

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Abstract

Seasonal and age-specific variations of cadmium (Cd) concentration in the digestive gland were investigated in the Japanese scallop *Patinopecten yessoensis* from Peter the Great Bay, Sea of Japan, with different degrees of Cd pollution. The seasonal changes in Cd concentrations of the digestive gland were inversely proportional to the dry weight of the gland. Concentrations of Cd and total Cd content (μg Cd per organ) increased with age (age-specific) to the same extent in contaminated and uncontaminated areas. There was also a strong positive correlation between Cd content in the whole digestive gland and shell weight and it is proposed that this relationship can be used as a new criterion for comparative evaluation of Cd levels in scallops from different areas We hypothesize that Cd is uptaken into scallops in proportion to the amount of calcium that absorbed through ion channels, and in addition, Cd in the digestive gland is in immobile forms (e.g. metal-rich granules) that accumulate with age. Moderate environmental pollution has no effect on the relationship between Cd content and shell size and the observed decrease in growth performance of the scallops from polluted areas may be due to other factors.

Keywords: Bioaccumulation; Heavy metals; Marine ecology; Molluscs; Pollution monitoring; Age-specific variation; Seasonal variation; Prognostic assessment

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1. Introduction

Many molluscs accumulate high concentrations of heavy metals in the digestive gland (Ahn, Lee, Kim, Shim, & Kim, 1996; Regoli & Orlando, 1994) and particularly scallops (Evtushenko, Luk'yanova, & Belcheva, 1990; Khristoforova, Kavun, & Makhnyr, 1989; Mauri, Orlando, Nigro, & Regoli, 1990; Ray, Woodside, Jerome, & Akagi, 1984). Therefore, monitoring metal concentrations in molluscan digestive glands has been proposed for assessing marine pollution (Bryan, 1973; Cossa, 1989; Regoli & Orlando, 1994). Since scallops are able to concentrate relatively high quantities of Cd compared to other bivalve molluscs (Gould & Fowler, 1991), they have been suggested as good indicators of available Cd (Bryan, 1973; Hungspreugs & Yuangthong, 1984; Luk'yanova & Martem'yanova, 1996). The Japanese scallop *Patinopecten yessoensis* has distinctively formed organs, such as gonads, digestive gland and kidney, which are relatively easy to dissect. The digestive gland has about 10% of the total body weight of the scallop and it can concentrate about 65% of total the Cd in mollusc soft tissues (Ikuta, 1985). Therefore the Japanese scallop is a convenient species for research on heavy metal cycling in the Sea of Japan.

It has been shown in many mollusc species that soft tissue heavy metal concentrations correlate with the total weight of the tissues. Moreover, this relationship is true for a relatively broad range of weight differences (from 10 to 400 fold) among molluscs (Boyden, 1977). However, the relationship between metal concentrations and animal size can be influenced by seasonal and geographical environment variations (Cossa, Bourget, Pouliot, Piuze, & Chanut, 1980; Kavun, 1994).

There appear to be species-specific differences in seasonal variability of metal concentrations in mollusc soft tissues. In some species metals in soft tissues decrease in concentrations during gonad growth and development, suggesting metal dilution by rapid growth (Boyden & Phillips, 1981; Fowler & Oregioni, 1976). In other studies such relationships have not been found (Bordin, McCourt, & Rodriguez, 1992; Talbot, 1986).

In this study two questions have been addressed: (i) What is the relationship between the seasonal changes in digestive gland condition and Cd concentrations? (ii) Does the Cd content of the scallop digestive gland correlate with metal contamination of the environment?

2. Materials and methods

The Japanese scallop *Patinopecten yessoensis* (Jay, 1857) is legally protected in the Russian Far East therefore sometimes the sample size (n) is limited by the quota, especially in case of the sites 2–4.We sampled scallops in various locations in the Peter the Great Bay of the Sea of Japan with different degrees of pollution (Fig. 1 and Table 1). The Site 1 is near the outfall of industrial and domestic wastes from Vladivostok city and is one of the most polluted areas in Peter the Great Bay (Vashchenko, 2000). Sites 2–4 are located in Far-Eastern State Marine Reserve.

Shell height, shell weight, total fresh weight of the molluscs, fresh weight of muscle and gonads were measured right after capture. The dry weight of the digestive gland was determined after drying it to a constant weight at 85 °C. The age of each 173 scallops was determined by detecting seasonal patterns of growth rings on its outer side of the upper valve (Silina, 1978). Every specimen was analysed individually. However, at Site 4 some immature scallops (from recently settled spat) were collected as well. Due

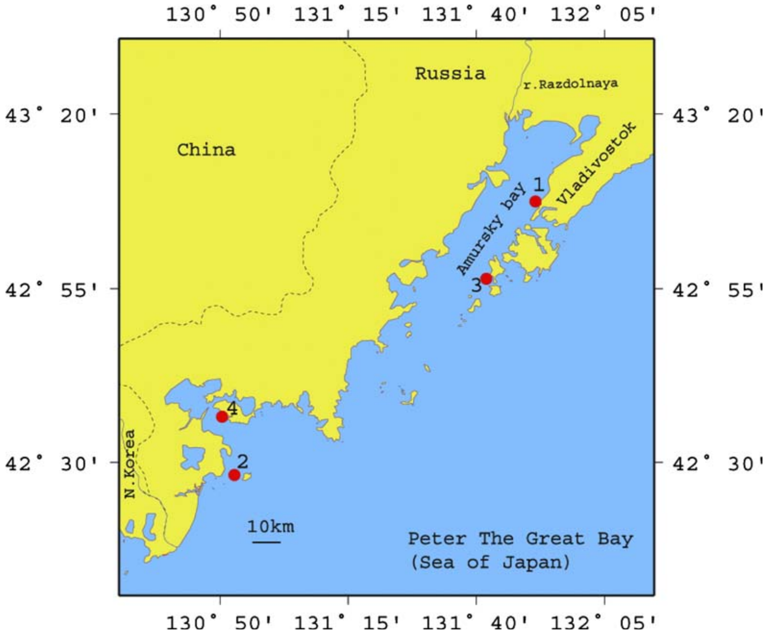

Fig. 1. Sampling sites in Peter The Great Bay of the Sea of Japan. For more details of the sampling sites see Table 1. The map has been created using USGS map generator (http://stellwagen.er.usgs.gov/mapit/).

Table 1
Sampling sites for scallops, *Patinopecten yessoensis*, morphometric data and cadmium concentration in these areas of Peter the Great Bay of the Sea of Japan

	Site 1 polluted	Site 2 unpolluted	Site 3 unpolluted	Site 4 unpolluted
Location and brief description	Vladivostok City, area of industrial and waste disposal	Furugelm Island, Far-Eastern State Marine Reserve, clearest area	Popov Island, Far-Eastern State Marine Reserve, clear area	Minonosok bight of Possjet Bay, farmed scallop in clear area
Scallop shell height (mm)	60–139	141–170	105–165	
Scallop age (year)	2–11	5–14	3–12	0.3–2
[Cd] sediments (μg/g of dry weight)	4.5–21.0	0.4–0.8	0.1–0.35	0.31
[Cd] water (μg/l)	0.7	0.02	0.14–0.25	0.04
Reference	(Tkalin et al., 1993; Shulkin & Kavun, 1995)	(Shulkin, 2001)	(Kovekovdova, 1993)	(Kovekovdova, 1993)
Sampling date	November 1998 (end) January (first half) 1999 March (beginning) 1999 June (last half) 1999 August (end) 1999	October 1998	June 1999	September 1999
N	113	18	19	135

to the small size of the spat, specimens of the digestive glands from 30 individuals were pooled for the analysis. Dried tissues (0.2–0.5 g) were homogenized in an agate mortar and mineralized in a mixture of 16 M HNO_3 and 11.3 M $HClO_4$ (3:1, v/v) in a teflon cylinder. After 24 h at room temperature the samples were digested at 90 °C until the solution became transparent.

The Cd content of the digestive gland of each scallop was analyzed individually using an atomic absorption spectrophotometer with flame atomization and deuterium background correction (Shimadzu AA-6800). Blanks (acids mixture) were processed with the sample at each digestion (1 blank + 10 samples). The detection limit in the 85 digested sample was 0.25 μg/g dry weight. Samples were always measured in triplicate; 8 the variability of reproducibility of the measurements was <20%.

ANOVA/MANOVA tests (STATISICA v6.0) were used to compare groups and reveal the effects of different factors (age, season, location). Both correlation and non-linear regression analyses were used to study the relationship between morphometric parameters and Cd contents using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego California USA). The *F*-test was used to compare entire regression curves using GraphPad Prism based on Motulsky (1995) and Sokal and Rohlf (1995).

3. Results

3.1. Age-specific size of scallops from different sites

The sizes of scallops (shell height and shell weight) of similar ages were not different among Sites 2–4 (unpolluted areas) (Fig. 2). Therefore these data were pooled for comparing with Site 1 (polluted area), and Site 1 scallops have shown significantly lower growth than those from Sites 2–4 (Fig. 2). However, age-specific sizes of 2-year-old scallops from Site 1 and Site 4 were not significantly different. Also, there was no seasonal variation in the height and weight of the shells from Site 1 (Fig. 2) (MANOVA; $P > 0.05$). Therefore data were presented as regression curves. It can be seen in Fig. 2 that the growth of scallops slows significantly after 3–4 years as scallops become mature, and the fastest changes of the shell height and shell weight are observed for ages below 3–4 years.

3.2. Effects of seasonal variation on soft tissues

Unlike shell parameters, the soft tissues were significantly affected by seasonal environment variation (MANOVA, $P < 0.05$, Fig. 3). All investigated soft tissues (total soft tissues, muscle, gonads and digestive gland) of scallops from Site 1 have shown a distinctive seasonal pattern in weight variation of organs with minimum variation during the summer period and maximum variation during the winter period (Fig. 3). The observed seasonal pattern of weight variation of soft tissues was identical in both wet and dry weights of the organs (Fig. 3). The seasonal weight variation is more profound in the scallops which mature (2- and 3-years old) as they prepare for the first spawning. For instance, in November at the beginning of gonad growth the 2- and 3-year-old scallops showed an almost twofold increase in gonad weight compared to the scallops sampled in August (period of sexual inertness) (Fig. 3B). For instance, maximum gonads' weight is observed in March with a subsequent decrease in June (spawning finished), but still the weights were higher than those in August. Seasonal variations of total soft tissue and muscle weight (Fig. 3A and C) were less expressed in 4-year-old scallops and older specimens compared to 2- and 3-year-old scallops, though the tendency is the same. The correlation coefficient between weights of gonads and digestive glands was $r = 0.85$ ($P < 0.0001$). Seasonal variations of

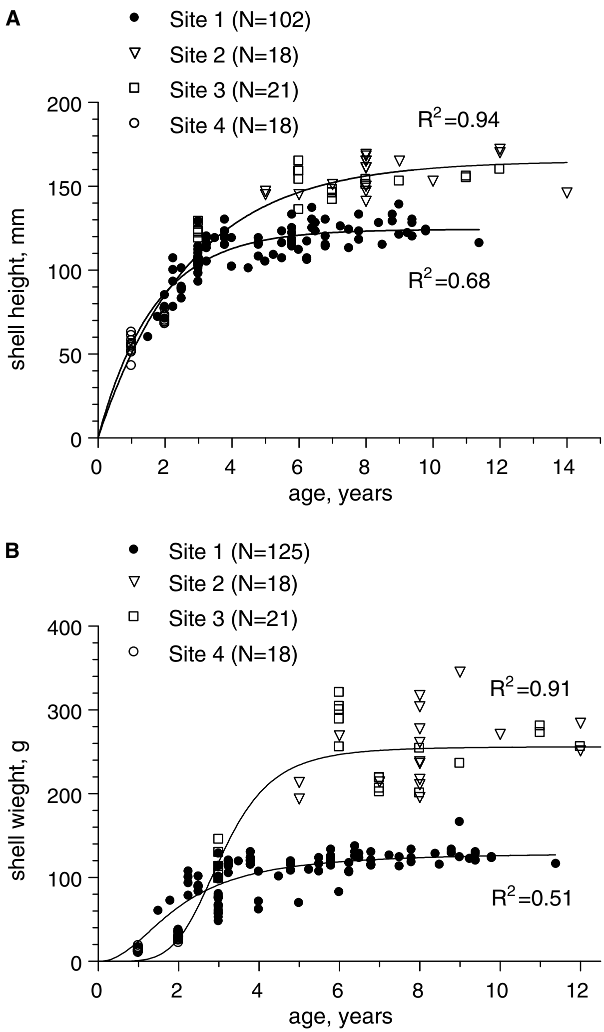

Fig. 2. Age-specific variation of: (A) shell height and (B) shell weight of *Patinopecten yessoensis* (polluted Site 1 versus pooled unpolluted Sites 2–4). Seasonal environment variation (followed only for Site 1, Table 1 and Fig. 3.) has no effect on these parameters (MANOVA, $P > 0.05$). F-tests for entire curves (includes all regression equation parameters) have shown significant differences between curves in both cases ($F_A = 48.52$ (8, 208), $P < 0.0001$; $F_B = 216.2$ (3, 176), $P < 0.0001$).

digestive gland weight decreases with scallop age in a similar manner to total soft tissue and muscle weight.

3.3. Age-specific and seasonal variations of Cd concentration and total Cd content in scallop digestive gland

The dry weight concentrations of Cd in the digestive gland increase significantly with age (Figs. 4 and 5). In addition, seasonal variations of Cd concentrations in the digestive gland were also observed (Fig. 4). For example, in winter when the weight of the digestive

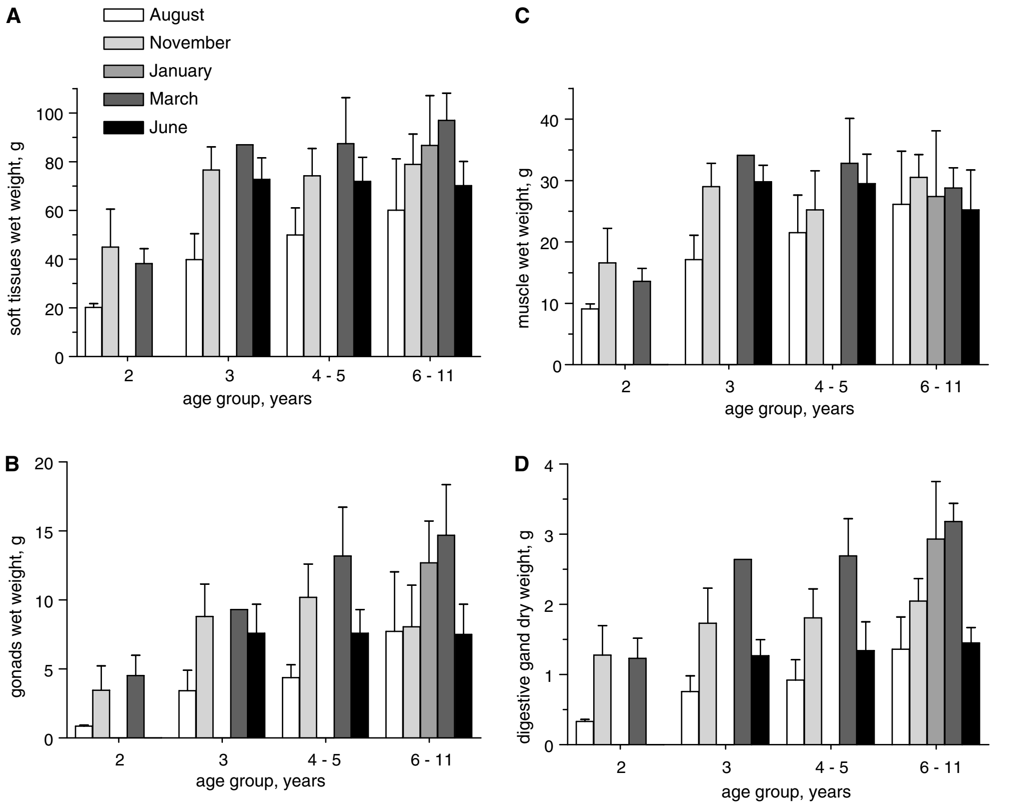

Fig. 3. Age-specific and seasonal variations of: (A) soft tissues, (B) gonads, and (C) muscle wet weight and (D) digestive gland dry weight of scallops *Patinopecten yessoensis* from Amursky Bay (Site 1). Values are mean ± S.D. ($n = 4$–18).

gland is highest (Fig. 3D; November–March, period of gametogenesis) Cd concentrations in the gland are two-fold lower than in summer (June–August, spawning or post-spawning inert period) (Fig. 4A). The effect of seasonal environment variation on Cd concentration in the digestive glad is also seen in Fig. 5A, which leads to relatively low R^2 for the regression curves. Aside from this relationship, total Cd content in whole digestive gland (μg Cd per organ) correlates strongly with age ($r = 0.81$; $P < 0.0001$), shell height ($r = 0.75$; $P < 0.0001$) and shell weight ($r = 0.85$; $P < 0.0001$; Fig. 5). Additionally, total Cd content in the whole digestive gland is less subject to seasonal environment variation as seen in Fig. 5B from the relatively high R^2 for the regression curves (compared to Fig. 5A).

As shell weight and total Cd content of the digestive gland vary little seasonally, they are the most useful measures for comparing populations with different anthropogenic effects, especially when collections are made in different seasons. Logarithmic transformation of these data leads to a linear relationship (with a positive slope significantly different from zero, $P < 0.0001$; Fig. 6). Further comparative analysis of linear regressions for unpolluted Sites 2–4 and polluted Site 1 (Tables 2 and 3) show that there is no significant difference between sampling sites for this relationship, either for slope nor Y-intercept (F-test, $P > 0.05$; Table 3). Therefore in Fig. 6 we have presented a regression line for the pooled data set.

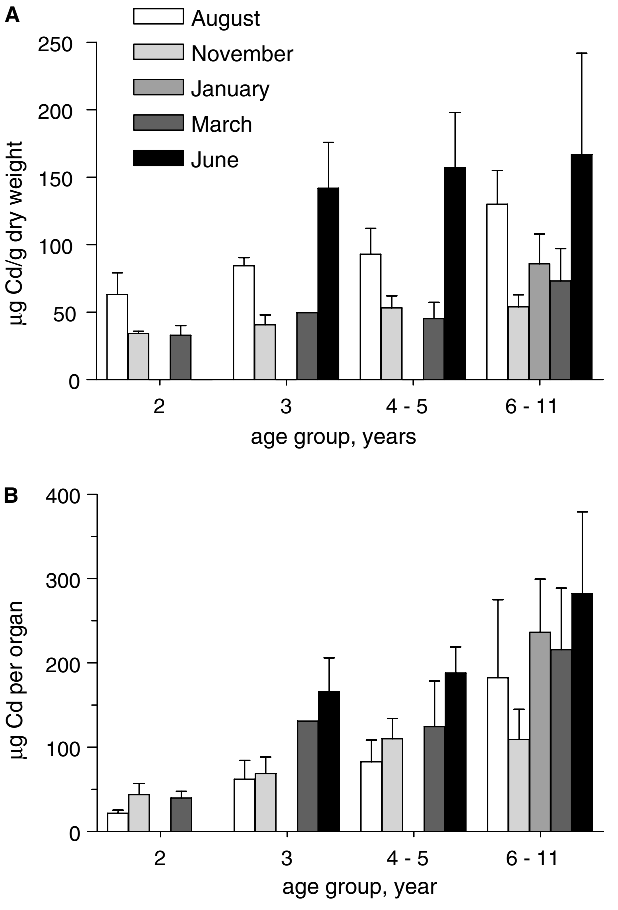

Fig. 4. Seasonal and age-specific variation variations of: (A) cadmium concentration in the digestive gland and (B) cadmium content in the whole digestive gland of scallops *Patinopecten yessoensis* from Site 1. Values are mean ± S.D. ($n = 4$–18).

However, surprisingly, both Cd concentration and Cd content in the whole digestive gland is higher in scallops from the unpolluted areas than from the polluted area (Fig. 5)! As was shown, the growth performance of scallops from the polluted area is lower than those from the unpolluted areas (Fig. 2). Consequently, it might be that this observed, but unexpected, difference results from environmental factors other than Cd that limit growth performance in the polluted ecosystems.

4. Discussion

4.1. Scallop morphometric parameters

Scallop growth significantly varies depending on environmental conditions (temperature, bottom type, salinity, oxygen availability, etc.) (Silina & Bregnam, 1986). High growth occurs in open water, with good water exchange and a constant salinity, for example at Sites

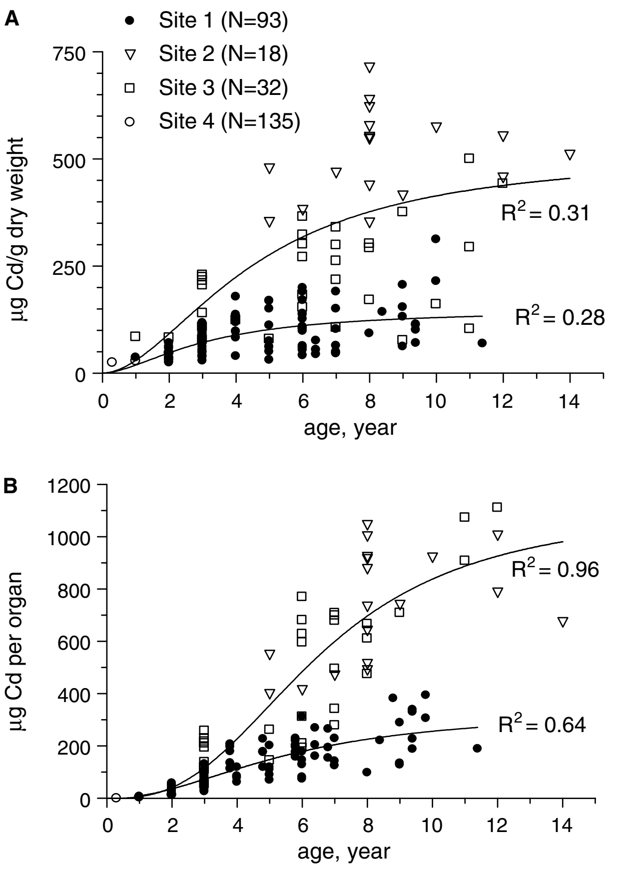

Fig. 5. Age-specific variation of: (A) cadmium concentration in digestive gland and (B) cadmium content in the whole digestive gland of *Patinopecten yessoensis* (polluted Site 1 versus pooled unpolluted Sites 2–4).

2 and 3 near islands in Peter the Great Bay. In contrast Site 1 is near the an industrial and domestic waste outfall from the city of Vladivostok and according to biological and chemical research is one of the most polluted areas in Peter the Great Bay (Vashchenko, 2000). Long-term records of slow growth of scallops at Site 1 is (Silina & Ovsyannikova, 1995), is consistent with our findings that shell height from Site 1 lower than those from unpolluted Sites 2–4 for the animals of the same age (Fig. 2).

This phenomenon might be explained by the fact that Site 1 is part of the shallow estuary of the Razdolnaya river (Fig. 1), where sometimes in August the water temperature can jump above 20 °C, salinity drops below 29%, and dissolved oxygen might drop below 5 mg ml^{-1}. Contamination from the outfall makes an additional contribution to these environmental limitations.

The weight of soft tissues varied seasonally with the reproductive cycle, with the most significant changes observed in the gonads and digestive gland (Fig. 3). Specifically, the weight of the gonads and digestive gland increases during the gametogenesis, decreases after spawning and remains low in post-spawning period of reproductive inertness. This finding is consistent with earlier studies. In Japanese waters similar results were reported

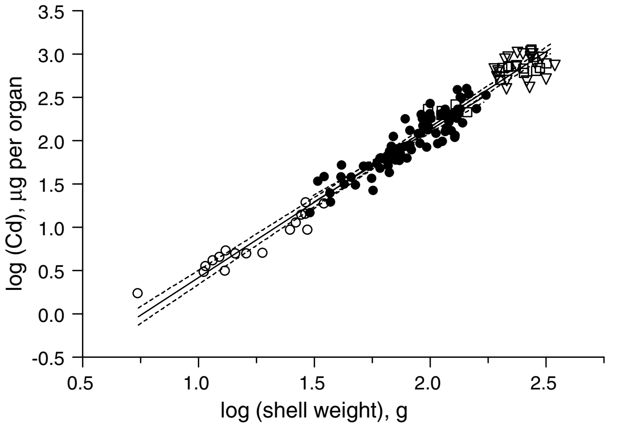

Fig. 6. A linear relationship between cadmium content in whole digestive gland and shell weight of *Patinopecten yessoensis*. Data from polluted Site 1 fits very well to the linear relationship derived for scallops from unpolluted Sites 2–4. Results of the regression analysis are presented in Tables 2 and 3. Dashed curves are 95% CI.

Table 2
Values (±S.E.M.) of linear regressions for the relationship between log(shell weight) vs. log(Cd per digestive gland) for unpolluted Sites 2–4 and polluted Site 1

Regression analysis	Polluted Site 1	Unpolluted Sites 2–4	Pooled Sites 1–4
Slope	1.655 ± 0.086	1.737 ± 0.036	1.725 ± 0.032
Y-intercept	−1.1487 ± 0.165	−1.317 ± 0.074	−1.311 ± 0.064
R^2	0.825	0.978	0.956

Table 3
F-test comparison for linear regressions between unpolluted Sites 2–4 and polluted Site 1

F-test	Slope	*Y*-intercept
F (df_n, df_d)	0.725 (1, 131)	1.316 (1, 132)
P	0.378	0.253

(Fuji & Hashizume, 1974). Weight decreases in the gonads (75%), digestive gland (60%), gills (10%), and mantle (10%) in the post-spawning period (April–May) for the 3-year-old scallop. In Peter the Great Bay the spawning occurs in May and June and according to our data digestive gland weight decreases by as much 50% in June and then decreases 20% from June to August. A cytological study has shown that after spawning the digestive gland undergoes significant morphological changes with atrophic and destructive tendencies (Usheva, 1990).

4.2. Age-specific and seasonal variations in Cd concentration

We found that Cd concentrations in digestive glands of adult scallops were age dependent. It is well known that the Cd concentration in soft tissues (such as mantle, kidney, and digestive gland) increase with growth in the Japanese scallop (Evtushenko et al., 1990; Khristoforova, 1996). This observation is in agreement with Boyden's (1977), that

Cd concentration in *Pecten maximus* did not depend on the size of specimens fewer than 2 g (dry weight), but older specimens had higher Cd concentrations. However, this relationship does not hold for all scallop species as Cd concentration in *Chlamis opercularis* does not depend on scallop size at all (Boyden, 1977). Studies of the Antarctic scallop *Adamussium colbecki* showed a reverse relationship between scallop size and Cd concentrations in soft tissue (Mauri et al., 1990).

We found that in the course of the year that Cd concentrations in the digestive gland correlate inversely with the organ's weight. Data of other authors on the same issue are contradictory, because the studies were mainly performed on the total soft tissue (pooled tissues). For example, in the oyster *Crassostrea gigas* which has been introduced to two areas with different levels of pollution indicated the reverse relationship between Cd concentration and body size (Boyden & Phillips, 1981). According to this data, the minimal Cd concentrations were observed during pre-spawning period in the oyster, when the body size was maximal. On the other hand, the maximal metal concentration was reported for the minimal body size. However, for *Macoma balthica* the maximum Cd concentration was reported during the maturation period, whereas the minimum Cd concentration was observed during the post-spawning period (Bordin et al., 1992). The mussel *Mytilus edulis* from an unpolluted area has shown no seasonal pattern of Cd concentration. That is why the authors assumed that the mollusc's condition has an effect on the Cd concentration (Boalch, Chan, & Taylor, 1981). *Modiolus kurilensis* exhibited a negative correlation between the weight of soft tissue and Cd concentration, but mussel *Crenomytilus grayanus* has shown that Cd concentration does not depend on variations of body size during the reproductive cycle (Shulkin & Kavun, 1994).

In any case, our results obtained for seasonal variations of Cd concentration in the digestive gland of the Japanese scallop are in agreement with earlier observations made by Luk'yanova and Martem'yanova (1996) for this species. They have studied a seasonal dynamic of Cd concentration in Japanese scallops from both polluted and unpolluted areas. In particular, Cd concentrations increase in digestive gland during the post-spawning period.

Surprisingly, we have observed that Cd content (in terms of both concentration and content) in scallops from unpolluted areas (Sites 2–4) was higher than those from the polluted area (Site 1, Fig. 5). We think that cadmium may be cycled into the organism as a function of the amount of calcium that it takes in through ion channels and then it is retained there in biologically inactive form. This would explain the increase of Cd content with scallop's age and shell size. It has been suggested that Cd enters aquatic organisms, at least in part, by the same uptake mechanism as Ca via calcium channels (Wright & Frain, 1981). The two elements have almost equal ionic radii, a similar number of electrons in the outer shell and equal charge, therefore the higher the calcium turnover the higher the Cd uptake, since competition between Ca and Cd for binding sites on calcium transporting proteins is indeed possible. Therefore, we think that the observed difference in Cd content in scallops from different sites indicates that the growth performance has a higher impact on Cd accumulation than age of the molluscs. It is known that growth performance of the Japanese scallop depends on many environmental factors such as temperature, bottom type (rocks, sands, pebbles, silts, and etc), oxygen availability, salinity, etc. It looks like the growth performance of the scallops in the polluted area is limited by some of these environmental factors which put scallops into a lower metabolic state. Indirectly it is proven by the smaller shell size of scallops in polluted areas (Fig. 2). From the other side, metabolically active scallops from unpolluted areas that are not limited by these factors "soak up" the trace concentrations of cadmium from the environment just because of high turnover of the metabolism and particularly due to higher calcium turnover.

4.3. Comparative analysis of Cd level in different scallop populations

Our results show that the seasonal variation in digestive gland weight correlates inversely with Cd concentrations. This can bring a significant error to comparative research when metal tissue concentrations are compared. As an alternative, our results have shown that total Cd content in a whole digestive gland is seasonally stable but tends to increase with age (Fig. 5). Our data is consistent with a relatively immobile nature of the accumulated cadmium in the digestive glad. That is, as the digestive gland decreases in the size all of its cadmium is retained, so cadmium content remains invariant. This is probably achieved via biological detoxification of cadmium (converting of it into bioinactive form), where metallothioneins (MT) and gluthatione system play crucial role (Chelomin, Belcheva, & Zakhartsev, 1998). Finally, accumulated cadmium is stored in form of metal-rich granules to sequester the cadmium away from metal-sensitive subcellular fractions (e.g. enzymes and organelles) and make it trophically unavailable to predators (Wallace, Lee, & Luoma, 2003; Wallace & Luoma, 2003). According to Wallace and Luoma (2003) such way of cadmium biological detoxification contributes to age and size dependent cadmium concentrations in bivalves.

Taking into account the above, we suggest using the relationship between total Cd content in a whole digestive gland and shell weight as a new criterion for comparative evaluation of Cd levels in scallops from different areas (Fig. 6). We assume that any deviation from this relationship can be considered as accumulation of excessive cadmium due to elevated levels of this element in bioavailable form in the environment. Partially, this approach has been inspired by Fischer (1983, 1986) where he has suggested using shell weight as an independent variable in relation to metal content in whole molluscs. It was shown that Fischer's approach provides a more reliable indication of metal bioavailability than the metal concentration in the environment itself, as shell weight remains independent from seasonal variations (Marigomez, Ireland, & Angulo, 1990; Regoli, 1998; Soto, Ireland, & Marigomez, 1997).

We have quantified the relationship (regression line, Fig. 6) between total Cd content in a whole digestive gland and shell weight for the Japanese scallop from the polluted Site 1. Surprisingly, the dependence of log(Cd content) in the whole digestive gland on log(shell weight) for scallops from Sites 2–4 was practically the same as it was for scallops from Site 1 (Tables 2, 3). It indicates that the level of accumulated cadmium in the scallop's digestive gland from all sites (1–4) was consistent with a natural one, although the Cd concentration in water and sediments of Site 1 was significantly higher. This could be due to two reasons: (i) either concentration of bioavailable forms of Cd in water (Site 1) is low, or (ii) the scallop is capable of regulating Cd accumulation in its tissues to some degree of environment pollution and this threshold was not overcome in Site 1. Studies of Cd accumulation in *C. grayanus* and *M. kurilensis* in coastal waters close to the city of Vladivostok showed that Cd accumulation in soft tissues occurs after the metal concentration threshold in the environment is surpassed (Shulkin, Kavun, Tkalin, & Presley, 2002). Thus, for both species Cd accumulation was not observed for values up to 3.1 μg Cd/g of dry weight in sediments. At Site 1, the Cd concentration in sediments is 4.5–21.0 μg Cd/g (dry weight of sediments) and 0.7 μg Cd/l in sea water, therefore it may be that these concentrations are not yet at the threshold for Japanese scallop and observed low growth performance at the polluted site is the result of effects of other environmental factors rather than a toxic effect of accumulated cadmium. To sum up the observations, using a new evaluation criterion we could not observe significant cadmium accumulation by scallops from moderately polluted area, although they show significantly lower growth performance, which is probably defined by other limiting environmental factors.

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