

Growth rate as a factor confounding the use of the dogwhelk *Nucella lapillus* as biomonitor of heavy metal contamination

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ABSTRACT: Growth rate of individually tagged dogwhelks *Nucella lapillus* (L.) was measured in free-living individuals at 3 sites of differing heavy metal contamination in the Firth of Clyde, west Scotland. Condition index (CI), concentrations of metals (Cd, Cu, Pb and Zn), metallothionein (MT), RNA (the RNA/protein ratio) and glycogen were also measured. In general, the marine environments of Gourock and Largs were contaminated with significantly higher tributyltin, Pb and Zn than Loch Fyne, as indicated by the results of imposex indices, and metal concentrations in transplanted polymer-ligands (Chelex® 100) and *Mytilus edulis*. Further, metal concentrations of native *M. edulis* (Pb and Zn) and *Semibalanus balanoides* (Cu) from Gourock were significantly higher than those from Loch Fyne. However, metal accumulation in the dogwhelks displayed a very different pattern. At a standard size (0.5 g wet soft-body weight), *N. lapillus* from Largs showed higher Cd, Cu and MT in their tissues than individuals from the other 2 populations. Levels of Pb and Zn were similar among the populations despite different concentrations in Chelex and mussels. Gourock dogwhelks showed similar levels of Cu and MT but lower Cd compared to those of Loch Fyne. These differences can be attributed primarily to differences in dogwhelk growth rate between sites. Gourock individuals had a higher CI and RNA/protein ratio in the foot muscle and grew faster (especially at small sizes), resulting in a tissue-dilution effect on metal and MT concentrations. In contrast, higher levels of Cd, Cu and MT in dogwhelks from Largs can be attributed to their growth rate being relatively slow compared to the rate of metal accumulation. Slow-growing individuals in Loch Fyne had relatively high Cd, Pb Zn and MT, although Loch Fyne has been regarded as a clean reference site. Among populations, differences in growth rate may be due to differences in prey availability, predation pressure, and/or genotype. The present results demonstrate that inter-site differences in growth rate can confound the use of the dogwhelks as a biomonitor of metals.

KEY WORDS: Dogwhelk · Growth · Metals · Metallothionein · RNA · Pollution

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INTRODUCTION

Marine intertidal gastropods such as the dogwhelk *Nucella lapillus*, the periwinkle *Littorina littorea*, and the limpet *Patella vulgata* meet most important require-

ments of the ideal biomonitor (Phillips 1980, 1990). These gastropod species are abundant throughout northern America and Europe, easy to identify and sample at all times of year, and large enough to provide sufficient tissue for analysis. They can tolerate wide ranges of contaminant concentration and of physico-chemical variables such as salinity, thereby permitting

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the design of transplant experiments and laboratory studies of contaminant kinetics. These gastropod species are also strong accumulators of certain metals such as Cd and Ag, although they can regulate essential metals like Cu and Zn (Langston & Spence 1995).

However, they may not fulfil the final criterion as ideal biomonitors because the metal concentration in their tissues is not always correlated with the average ambient bioavailable metal concentration (Phillips 1980, 1990). Ireland & Wootton (1977) noted that contamination profiles generated from the analysis of *Nucella lapillus* and *Littorina littorea* at 9 sites on the coast of Wales did not match the relative levels of metals in local seawater. Other studies using *L. littorea* as a biomonitor for trace metals reached a similar conclusion (Bryan et al. 1983, Leung & Furness 1999a). The different contamination profiles displayed by gastropod species might be explained by an inconsistent relationship between the comparative levels of metals in solution and metals associated with particulates (Phillips 1990). Alternatively, Bryan et al. (1983) suggested that such discrepancies might be due to different concentrations of metals in the diet consumed by the gastropods, as well as different food types and food availabilities amongst areas. Another possibility is that the discrepancies in the metal concentration profiles could be due to differences in growth rate among populations, as suggested by differences in the condition index of *L. littorea* between study sites (Leung & Furness 1999a). However, the last hypothesis has not been tested by field experiments.

It has been widely accepted that weight-specific metal concentration (e.g. mg g⁻¹ dry tissue weight) in a biomonitor is affected by growth rate (Phillips & Rainbow 1993, Langston & Spence 1995, Rainbow 1996). In theory, the concentration of a metal will increase with increasing age and weight provided that growth is slow relative to the rate of accumulation of that element. Alternatively, if growth is rapid compared to metal accumulation, the observed concentration of the element will decrease with increasing age and weight, even though the overall metal content may be increasing (Phillips & Rainbow 1993). The latter phenomenon is called the 'tissue-dilution effect'. If this effect is substantial, any biomonitoring programme should incorporate growth measurement of the biomonitors, as growth rate may vary amongst individuals and populations. Nonetheless, reports on biomonitoring of trace metals seldom consider the possibility of intraspecific differences in growth of biomonitors between study areas, a priori assuming that all populations have a similar growth rate. If growth rate is a predominant factor affecting the metal concentration in a biomonitor, the conventional approach without taking growth into account will lead to a potential pitfall in biomoni-

toring. For example, a population of biomonitors growing fast in a metal-contaminated area may present similar or even lower metal concentrations than in animals with a slow growth rate in clean areas. Such an effect might be anticipated, since metal concentration is often associated with organic enrichment.

Both food type and food availability have significant effects on the growth rate of marine gastropods, and thus may affect the concentrations of metals and metallothionein (MT) in tissues. In dogwhelks, feeding mussels alone or combined with barnacles promotes better growth than feeding barnacles alone (Etter 1996). In the laboratory, adult dogwhelks fed with mussels or barnacles and exposed to Cd had a significantly lower Cd concentration in their tissues than that in starved Cd-exposed dogwhelks, consistent with a tissue-dilution effect occurring in fed individuals (Leung & Furness 2001). These laboratory results invite experiments to determine whether they apply to wild populations.

Apart from trace metals, concentrations of biomarkers such as MT or MT-like proteins, and glycogen stores may also be influenced by growth. A key objective of the present study was to examine whether growth influences metal accumulation, MT synthesis and glycogen stores of *Nucella lapillus* under natural environmental conditions. We investigated *in situ* growth rates, nutritional status (through the RNA/protein ratio) and metal contamination of 3 different intertidal populations of *N. lapillus* from 3 areas of the Firth of Clyde, western Scotland, with differing levels of metal contamination. Based on this comprehensive biomarker approach, accumulation and toxicity of trace metals in these populations of *N. lapillus* were evaluated.

MATERIALS AND METHODS

Description of study sites. The Firth of Clyde is a semi-enclosed sea area on the west coast of Scotland which receives direct inputs of contaminants, via rivers, pipelines, sea dumping, farm and fish-farm waste discharges (Muller 1998). The major inflow of freshwater to the Clyde Sea is from the Clyde Estuary into which drain the effluents of approximately half of Scotland's population and industry (Fig. 1; Thomason et al. 1997). Population samples of *Nucella lapillus* were collected from Gourock, Largs and Loch Fyne, from enclosed and protected rocky intertidal shores (Fig. 1). Gourock is situated in the Clyde Estuary and is regarded as a metal-contaminated area, in particular Pb and Zn (Miller 1986, Balls et al. 1997). Loch Fyne is in a relatively remote area, mainly serving forestry, extensive grazing, aquaculture and recreation pur-

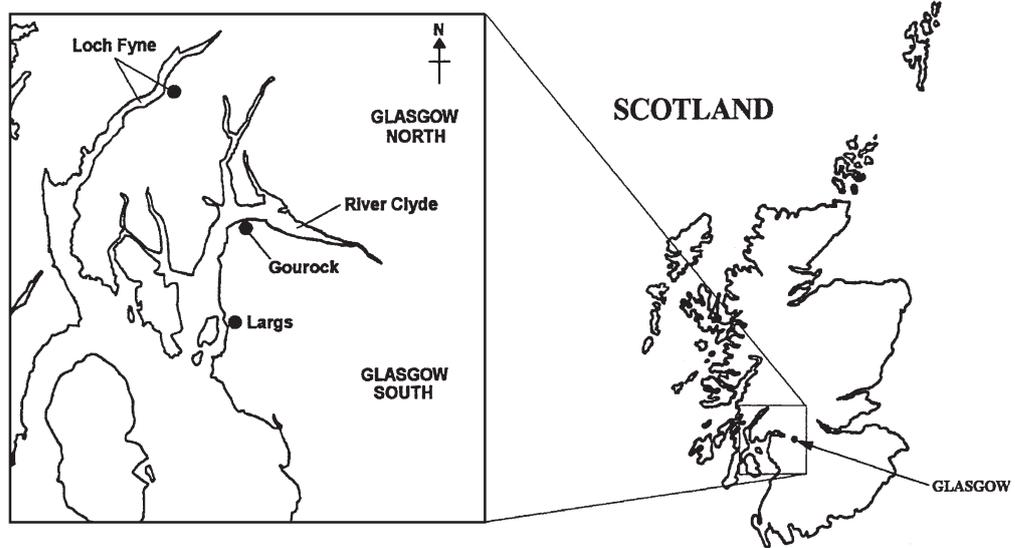


Fig. 1. Map of Clyde Estuary, showing the study sites

poses, and therefore is expected to be relatively free from metal contamination, whilst Largs lies in the Clyde Sea and is thus likely to be of intermediate pollution status.

Sampling. Different sizes of the dogwhelk *Nucella lapillus* were collected at low tide from all these areas from 24 to 30 May 1998, returned to the laboratory and maintained in water tanks supplied with circulating seawater (35 psu, 10°C). Amongst the areas, Gourock had the highest abundance of *N. lapillus*, followed by Largs, while Loch Fyne had the lowest abundance, indicated by the catch per unit effort (Table 1). At the time of collection a note was made of the presence or absence of the 2 barnacles *Semibalanus balanoides* and *Chthamalus* spp., and also of the abundance of the mussel *Mytilus edulis* (Table 2), all of which are regular prey of dogwhelks (Moore 1936, 1938b, Burrows & Hughes 1990). In order to estimate the metal concentrations in the foods of the dogwhelks, native *S. balanoides* and *M. edulis* were also collected from each site where possible, and stored at -25°C pending further metal analyses.

Estimation of *in situ* growth rate. After acclimation in the aquarium for 1 to 2 d, each individual was tagged with a waterproof label (4 × 2 mm²) using

super-glue (151 Super Glue, SB Ltd, UK). Shell length was measured with vernier callipers (±0.1 mm). Shell and live body mass were estimated at the beginning of the experiment using the methods of Palmer (1982). The dogwhelks were maintained immersed for at least 24 h to allow air bubbles in the mantle cavity to dissolve completely. Each dogwhelk was then transferred to a weighted cradle, suspended from the arm of a balance (±1.0 mg) by a stainless steel rod, and immersed in a small rectangular tank of seawater (length × width × height: 16.3 × 6.3 × 10.0 cm³; volume of seawater at 35 psu = 860 ml). The measured weight largely reflects the mass of the shell, as the density of body tissues is close to that of seawater (Burrows & Hughes 1990). After weighing in water, each snail was dried and extra-visceral water was removed by pressing an absorbent tissue firmly against the withdrawn foot until no further fluid penetrated the tissue. It was then left to dry for about 1 h before weighing in air. The true mass of shell for each population was estimated using regressions of dry shell mass on both immersed total mass under water and the total mass in air (Table 3). The soft-body weight was obtained by subtraction of the estimated shell mass from the total mass in air. For each population, different sizes of *Nucella lapillus* (n =

23 to 44) were put through the same procedures and sacrificed to establish calibration regressions for each site (Table 3). According to the method described by Davenport et al. (1998), the condition index (CI) of each dogwhelk was also calculated using the following equation:

Table 1. *Nucella lapillus*. Date of release and sampling of tagged dogwhelks from different study sites in 1998, with numbers and percentage recovered. CPA: catch per unit effort (nos. h⁻¹ person⁻¹)

Area	Release date	No. released	CPA	Sampling date	Exposure time (d)	No. re-collected	% recovery
Gourock	4 June	355	142	20 July	47	30	8.4
Largs	3 June	288	115	15 August	73	17	5.9
Loch Fyne	30 May	89	30	21 July	53	12	13.5

Table 2. Observed characteristics of food (mussel and barnacles) availability in the study sites. +: presence doubtful or else absent in immediate locality; ++: present in small numbers; +++: present in large numbers

Area	<i>Mytilus edulis</i> abundance	<i>Semibalanus balanoides</i>	<i>Chthamalus</i> spp.
Gourock	++	Present	Absent
Largs	+	Present	Absent
Loch Fyne	+++	Present	Present

Table 3. *Nucella lapillus*. Morphometric relations used in growth estimations. Shell mass (g) estimates of destructively sampled *N. lapillus*: shell dry mass (Y) from immersed whole weight (X) and weight (g) in air (Z)

Study site	(n)	Regression equation	R ²
Gourock	(44)	$Y = 0.2330X + 0.5557Z - 0.0562$ ($F_{2,41} = 1638.13, p < 0.001$)	0.9876
Largs	(39)	$Y = 0.2715X + 0.6233Z - 0.0064$ ($F_{2,36} = 1460.31, p < 0.001$)	0.9878
Loch Fyne	(23)	$Y = 0.6877X + 0.4658Z - 0.0001$ ($F_{2,26} = 575.25, p < 0.001$)	0.9924

$$CI = \frac{\text{wet soft-body wt}}{\text{wet soft-body wt} + \text{dry shell wt}} \times 100 \quad (1)$$

The tagged and weighed snails were then released into their original habitats within 5 to 7 d. After 6 to 10 wk, the tagged individuals were re-collected (Table 1). The recapture rate of tagged individuals was 5.9 to 13.5%. Non-tagged snails were also collected and included in metal and biochemical analysis (there was no statistical difference in any parameter between tagged and non-tagged individuals in each area: Student's *t*-tests or ANCOVA; $p > 0.05$). *Nucella lapillus* shells were removed using a vice. The wet weight of the soft-body was measured using an electronic balance after blot-drying with an absorbent tissue. The soft-body tissues were stored at -25°C to await further analysis. Growth rate for individual dogwhelks was calculated by the difference between the final and initial estimated soft-body weight and expressed as $\text{mg g}^{-1} \text{d}^{-1}$.

Metal analysis. The soft-body of each live dogwhelk was dissected into foot muscles, upper and lower parts of digestive gland/gonad complex, and the gland of Leiblein ($n = 30$ for each site). The tissues of digestive gland/gonad complex (upper part) of the dogwhelks were dried at 60°C for at least 96 h until constant mass was achieved. They were then digested in concentrated HNO_3 for 24 h at room temperature followed by boiling for at least 2 h until a clear solution was obtained. The concentrations of Cd, Cu, Pb and Zn

were determined using a Philips PU9200 atomic absorption spectrophotometer (AAS) with deuterium background correction, and expressed as $\mu\text{g g}^{-1}$ dry tissue weight. Accuracy was regularly checked by including a standard reference material (dogfish muscle, DORM-1, from the National Research Council, Canada) within batches (Table 4). For each site, soft tissues of 15 native mussels, *Mytilus edulis* (shell length = 31.3 ± 2.1 mm) and 6 pooled samples of 15 native barnacles *Semibalanus balanoides* (diameter = 3.8 ± 0.5 mm) were dissected, dried, acid-digested and analysed for Cd, Cu, Pb and Zn, following the above procedures.

Quantification of MT and glycogen. The gland of Leiblein of *Nucella lapillus* was analysed for MT concentration because it is the most important and sensitive site for Cd accumulation and Cd-MT induction (Leung & Furness 1999b). This is attributed to the primary function of the Leiblein gland, which produces, stores and secretes an enzyme (Calcase) and EDTA-like chemicals for the dissolution drilling mechanism (Purchon 1977, P. S. Rainbow pers. comm.). The weighed whole Leiblein gland was homogenised with 0.4 ml of 0.25M sucrose using an Ultraturax (T25 Janke & Kunkel, IKA Labortechnik) at 4°C . The homogenate was centrifuged at $20000 \times g$ for 20 min at 4°C . Aliquots of 300 μl supernatant were analysed for MT content using the silver saturation method (Scheuhammer & Cherian 1991, as modified by Leung & Furness 1999b). Briefly, samples were incubated with 0.4 ml glycine buffer (0.5M, pH 8.5) and 0.5 ml of 20 mg l^{-1} silver solution for 20 min at 20°C to saturate the metal-binding sites of MT. Excess silver ions were removed by the addition of 100 μl bovine red blood cell hemolysate to the assay tubes followed by heat treatment in a water bath (100°C for 10 min). The heat treatment caused precipitation of silver-bound haemoglobin and other proteins, except for MT, which is heat-stable. The denatured proteins were removed by centrifugation at $1200 \times g$ for 10 min. The hemolysate addition, heat treatment and centrifugation were repeated 3 times in each sample. Finally, the supernatant was centrifuged at $20000 \times g$ for 10 min. The

Table 4. Comparison of metal concentrations ($\mu\text{g g}^{-1}$ dry wt; means \pm 95% CI) in standard reference material, dogfish muscle, DORM-1, certified by the National Research Council of Canada, and analytical results from the current study

Metal	Certified	Current study ($n = 8$)
Cd	0.086 ± 0.012	0.072 ± 0.010
Cu	5.22 ± 0.33	4.87 ± 0.19
Pb	0.40 ± 0.12	0.40 ± 0.07
Zn	21.3 ± 1.0	22.6 ± 2.9

amount of silver metal in the final supernatant fraction, which was proportional to the amount of MT present, was determined using the Philips PU9200 AAS. Assay tubes containing purified horse kidney MT obtained from Sigma Chemicals in a range of concentrations from 2 to 20 μg underwent the same process in order to establish a calibration curve ($\mu\text{g Ag ml}^{-1}$ vs $\mu\text{g MT ml}^{-1}$) for MT quantification.

For glycogen analysis, 50 to 100 mg of the digestive gland/gonad complex (lower part) or foot muscle tissues was dissolved in 0.4 ml 30% KOH at 90°C for 30 min. After cooling in ice, 1 ml of absolute alcohol was added to the tissue solution, mixed and kept at 4°C for 2 h. It was then centrifuged at $3\,000 \times g$ for 10 min. After removal of the supernatant, the pellet was re-dissolved in 1 ml distilled water. Subsequently, glycogen concentrations in these solutions were determined in triplicate by utilising the anthrone reagent (Seifter et al. 1950), with comparison against multiple glycogen standards. The results of MT and glycogen were expressed as μg or mg g^{-1} wet tissue weight.

RNA and RNA/protein ratio. Frozen foot muscle (~100 mg) was homogenised in 3 ml of ice-cold 0.2 M perchloric acid (PCA) in a 5 ml test tube for 20 s and transferred to a clean, 15 ml polypropylene centrifuge tube on ice. The test tube was washed twice for 20 s with 1 ml PCA which was added to the centrifuge tube. The homogenate (4 ml) was centrifuged at $6\,000 \times g$ for 10 min at 4°C and the supernatant discarded. The pellet was re-suspended (by sonication) and washed in 3 ml 0.2 M PCA twice and then re-suspended in 4.5 ml distilled water. The suspension was gently mixed with 0.5 ml of 3M NaOH and incubated at 37°C for 1 h, with regular shaking to ensure solubilisation. (The alkaline condition re-dissolved proteins: separated rRNA from ribosomal protein and totally solubilised this RNA.) Samples were allowed to cool and mix. Two 50 μl aliquots were stored at -20°C for later Lowry protein assay (Lowry et al. 1951, as modified by Schacterle & Pollock 1973). 0.9 ml of 20% PCA (3.4 M) was added to the remaining 3.9 ml samples, mixed and centrifuged at $6\,000 \times g$ for 10 min at 4°C. (This caused all the protein and DNA to precipitate but left protein-free RNA in solution.) The supernatant was stored on ice in labelled 15 ml polypropylene centrifuge tubes for later RNA assay, using Orcinol reagent. A series of RNA (yeast RNA from Sigma Chemicals) standards (0 to 50 $\mu\text{g RNA ml}^{-1}$) were prepared with 0.5 M HCl. Orcinol reagent was prepared by dissolving 120 mg Orcinol per 20 ml FeCl_3/HCl solution (20 mg FeCl_3 dissolved in 100 ml concentrated HCl). One ml of sample or standard was pipetted into a glass test tube and mixed with 1 ml 0.5 M HCl and 2 ml Orcinol reagent in a fume cupboard. The assay tubes were heated at 100°C in a water bath for 35 min in a fume cupboard, and then

allowed to cool to room temperature. Absorbance of each sample or standard was read at 665 nm. The results of RNA were expressed as $\mu\text{g RNA g}^{-1}$ dry tissue weight and $\mu\text{g RNA mg}^{-1}$ protein.

Imposex determination and tin measurement. On the date of re-sampling, non-tagged adult dogwhelks ($n = 40$) were collected at the same areas for imposex determination. The relative penis size index (RPSI) is the mean bulk of the female penis (length^3) expressed as a percentage of the mean bulk of the male penis (length^3) in a sample (Davies et al. 1997). The vas deferens stage index (VDSI), which is an index for the individual stage of the development of a vas deferens in the female, was determined as described by Gibbs et al. (1987). For each site, 5 adult females were randomly selected and their digestive glands were dissected for tin quantification. The tissue samples were dried and acid-digested with concentrated HNO_3 as described above. The total concentration of tin in these samples was then determined using an inductively-coupled plasma mass spectrometry (ICP-MS) (Perkin-Elmer Elan 5000) and expressed as ng g^{-1} dry tissue weight.

Trace metals monitored by Chelex® 100 and mussels. To compare the average environmental trace metal levels in these 3 areas, Chelex tubes and mussels, *Mytilus edulis* were deployed. Chelex resins mainly accumulate the ionic fraction of metals, while *M. edulis* accumulates both particulate and ionic forms. Mussels *M. edulis* (48 ± 1 mm in shell length) were collected from Loch Fyne and acclimated in an aquarium with circulating seawater at 35 psu and 10°C for 5 to 10 d. During acclimation, 30 mussels and 15 Chelex tubes (Wu & Lau 1996) were placed in cages; 3 such cages were prepared and deployed in the study areas by anchoring them with rocks. They were recovered after 6 wk exposure. Shell length and dry biomass of the mussels were measured in order to determine the condition factor (mg mm^{-1}). The concentrations of Cd, Cu, Pb and Zn in the Chelex and mussels were determined using an ICP-emission spectrometry (ICP-ES) (Perkin-Elmer Plasma 1000) and graphite furnace atomic spectrometry (Thermal Jarrell Ash Smith-Hieftie 12), respectively, after acid-digestion with concentrated nitric acid (Wu & Lau 1996). Results were expressed as $\mu\text{g g}^{-1}$ dry weight.

Statistical analysis. All data were natural-log-transformed, except growth rate (there were negative values, and thus normal data were utilised). Normality and homogeneity of variances of the data were checked using the Kolmogorov-Smirnov test and Bartlett's test, respectively. General linear models (GLM) were used to test the effects of snail size and sampling site on all parameters. There were no size effects on MT, glycogen and RNA/protein data. Therefore, comparisons between these size-independent

data were made based on mean or median values. Nevertheless, analyses of growth, metal, CI and shell weight-length data were made using analysis of covariance (ANCOVA in GLM) using wet soft-body weight as covariate. There were no significant differences in any parameters between tagged and non-tagged individuals (ANCOVA was used to test size-dependent data; Student's *t*-tests were used to test size-independent data; $p > 0.05$). Therefore, 18 and 13 non-tagged dogwhelks of different sizes were added to Largs and Loch Fyne tagged samples, respectively, and analysed together with the tagged individuals (total $n = 30$). For parametric data with similar standard deviations, differences in size-independent parameters between areas were compared using a 1-way analysis of variance (ANOVA), with subsequent comparison between individual means using a Tukey-Kramer multiple comparison test. For non-parametric data or data with different SDs, differences in the size-independent parameters between sites were compared using Kruskal-Wallis tests, with subsequent comparison between individual means using a Dunn's multiple-comparison test. Correlations between different parameters were examined using Pearson's correlation analysis. Partial correlation analysis was also performed on the concentrations of MT and MT-inducing metals (including Cd, Cu and Zn) or the RNA/protein ratio with a correction for size. Concentrations of metals ($\mu\text{g g}^{-1}$ wet wt) were standardised at a size of 500 mg wet soft-body weight using the regression between the metal concentration and wet soft-body weight. ANOVA was applied to test the differences between the mean values of metal concentrations, with a subsequent comparison between individual means using the Tukey-Kramer multiple-comparison test. Statistical significance was defined as $p < 0.05$. All statistics were run on standard software packages (SPSS for Windows, Release 7.5.1, 1996 and Graph Pad Prism™, Version 2.0, 1995).

RESULTS

General metal contamination profiles

The highest imposex index (RPSI or VDSI) was observed in *Nucella lapillus* from Gourock followed by Largs (Table 5). Among dogwhelks from Loch Fyne, very few presented 1 to 2 stages of the vas deferens sequence, so that RPSI approached zero. The results of imposex indices were also in good agreement with the results of Sn concentrations in the digestive glands of female dogwhelks (Table 5), indicating that *N. lapillus* from Loch Fyne had significantly lower tin (Sn) accumulation than those from the other 2 sites. These

Table 5. *Nucella lapillus*. Imposex indices (RPSI and VDSI; $n = 40$; means \pm SD) and tin concentration in the digestive glands ($n = 5$; all females) of adults collected from the study sites. For VDSI and tin concentrations, values bearing same letter are not significantly different (Tukey-Kramer multiple-comparison test, $p > 0.05$). RPSI: relative penis size index; VDSI: vas deferens stage index.

Area	Shell length (mm)	RPSI	VDSI	Tin (ng g^{-1} dry wt)
Gourock	32.2 \pm 3.3	0.82	3.25 \pm 1.14 ^a	324.1 \pm 76.6 ^a
Largs	31.6 \pm 4.0	0.03	1.86 \pm 1.51 ^b	338.1 \pm 38.3 ^a
Loch Fyne	34.7 \pm 3.1	0.00	0.50 \pm 0.90 ^c	208.4 \pm 50.7 ^b

results indicated that Gourock was highly contaminated with endocrine-disrupting chemicals, in particular organotin compounds (e.g. tributyltin); Largs was in intermediate pollution status while Loch Fyne showed very little contamination with these endocrine-disrupting chemicals.

There was no significant difference in the condition factor of transplanted *Mytilus edulis* among sites ($F_{2,37} = 2.39$, $p = 0.106$; Table 6), suggesting that the growth rates of the mussels were similar. There was no significant difference in Cd concentration of transplanted Chelex or mussels among sites, but Pb and Zn accumulations were generally lowest at Loch Fyne, indicating that this was the less metal-contaminated of the 3 sites (Table 6).

Based on our field observations, all sites were covered with the barnacle *Semibalanus balanoides*, while the mussel *Mytilus edulis* was more frequently found in Gourock and Loch Fyne but not in Largs (Table 2). As food consumption could be an essential pathway of metal accumulation by dogwhelks, we determined the concentrations of various metals in native *M. edulis* and *S. balanoides* from the study sites (Table 6). Alongside the transplanted Chelex and mussels, native *M. edulis* of Gourock also exhibited significantly higher Pb and Zn concentrations than those from Loch Fyne (Table 6). Further, the Cu concentration of native *S. balanoides* from Gourock was also significantly higher than that of the barnacles from Largs and Loch Fyne (Table 6).

Characteristics of *Nucella lapillus* populations

The size-frequency distribution of each population was constructed based on the shell length of specimens used for tagging (Fig. 2). All populations showed a continuous size structure. Gourock's population peaked at 18 to 22 mm and 26 to 28 mm; the size structure of the Largs population was similar, with highest abundance at 26 to 28 mm. Dogwhelks from Loch Fyne

Table 6. Concentrations (means ± SD) of various metals in deployed Chelex® 100, transplanted mussels *Mytilus edulis*, native *M. edulis* and *Semibalanus balanoides* in different study areas. Values of condition factor (CF) of transplanted mussels from each site are also shown (n = sample size). For concentrations of each metal in Chelex, mussels or barnacles, values with same letter are not significantly different (p > 0.05; for all cases, Tukey-Kramer multiple-comparison tests were used, except that Student's t-tests were used for data of native mussels). nd: not detected

	(n)	Concentration (µg g ⁻¹ dry wt)				CF
		Cd	Cu	Pb	Zn (mg mm ⁻¹)	
Transplanted Chelex® 100						
Gourock	(12)		2.69 ± 1.04 ^a	0.81 ± 0.97 ^b	2.49 ± 0.05 ^b	18.78 ± 9.75 ^a
Largs	(10)		2.27 ± 0.64 ^a	1.92 ± 0.52 ^a	4.34 ± 1.66 ^a	6.42 ± 2.55 ^b
Loch Fyne	(9)		3.19 ± 1.79 ^a	0.97 ± 1.40 ^{ab}	1.20 ± 0.53 ^c	9.64 ± 5.00 ^b
Transplanted <i>M. edulis</i>						
Gourock	(10)		2.70 ± 3.39 ^a	8.99 ± 4.87 ^a	6.98 ± 2.48 ^a	110.50 ± 62.17 ^a
Largs	(15)		1.56 ± 0.52 ^a	8.74 ± 6.56 ^a	5.12 ± 2.63 ^{ab}	85.36 ± 19.58 ^{ab}
Loch Fyne	(15)		1.31 ± 0.39 ^a	6.53 ± 0.72 ^a	3.64 ± 3.84 ^b	64.94 ± 12.72 ^b
Native <i>M. edulis</i>						
Gourock	(15)		0.57 ± 1.27 ^a	6.90 ± 1.20 ^a	7.43 ± 4.91 ^a	80.03 ± 24.69 ^a
Largs			<i>M. edulis</i> not found at this study site			
Loch Fyne	(15)		0.65 ± 0.64 ^a	4.96 ± 1.11 ^a	0.90 ± 1.79 ^b	47.72 ± 17.59 ^b
Native <i>S. balanoides</i>^a						
Gourock	(6)		2.03 ± 0.96 ^a	36.07 ± 5.24 ^a	nd	1255.9 ± 203.7 ^a
Largs	(6)		2.01 ± 0.55 ^a	23.49 ± 2.45 ^b	nd	996.9 ± 144.8 ^b
Loch Fyne	(6)		2.33 ± 0.54 ^a	26.68 ± 2.60 ^b	nd	1015.9 ± 131.3 ^{ab}

^aEach sample was pooled data of 15 individuals

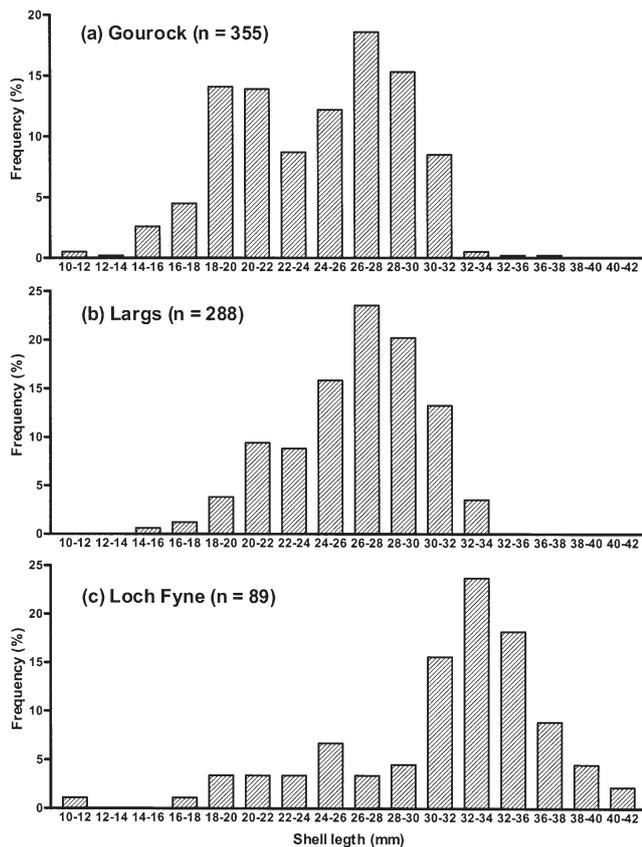


Fig. 2. *Nucella lapillus*. Size-frequency distribution of the 3 populations

showed a different size distribution, with a high abundance of large individuals (30 to 36 mm), the size class 32 to 34 mm being the most abundant. The maximum size in this population was 40 to 42 mm, much greater than that of the populations from Gourock (32 to 34 mm) and Largs (36 to 38 mm).

The dogwhelks of Gourock had significantly higher CIs than those of Largs, while the lowest CIs were observed in Loch Fyne dogwhelks (Fig. 3; Table 7; ANCOVA: p < 0.001). The lower CIs in Largs and Loch Fyne individuals could be partially explained by a heavier shell weight, especially in large dogwhelks (Fig. 4; Table 7; ANCOVA: p < 0.001). The results of the *in situ* growth study showed that *Nucella lapillus* from Gourock and Loch Fyne grew faster than those from Largs under natural environmental conditions (Fig. 5; Table 7). At small sizes (<0.7 g wet soft-body wt), Gourock's population grew faster than the dogwhelks from Loch Fyne, but the latter population had a higher growth rate at large sizes (>1 g) (Table 7; interaction p < 0.001). During the study period, weight loss was observed in most dogwhelks from Largs, and in large individuals from Gourock.

Metals and MT in *Nucella lapillus*

In general, the concentrations of trace metals in the digestive gland/gonad complex of *Nucella lapillus*

Table 7. *Nucella lapillus*. Analyses of covariance of condition index, shell weight and growth rate versus area and size (wet soft-body mass) where size is the covariate. *p < 0.05; **p < 0.01; *** p < 0.001

	Condition index			Shell weight			Growth rate		
	df	MS	p	df	MS	p	df	MS	p
Regression	3	2.181	<0.001***	3	12.561	<0.001***	3	2558.1	<0.001***
Area	2	1.245	<0.001***	2	1.483	<0.001***	2	1310.8	<0.001***
Size	1	3.335	<0.001***	1	30.988	<0.001***	1	6605.2	<0.001***
Area × size	1	1.392	<0.001***	1	19.434	<0.001***	1	7802.3	<0.001***
Residual	102	0.023		102	0.028		55	142.1	

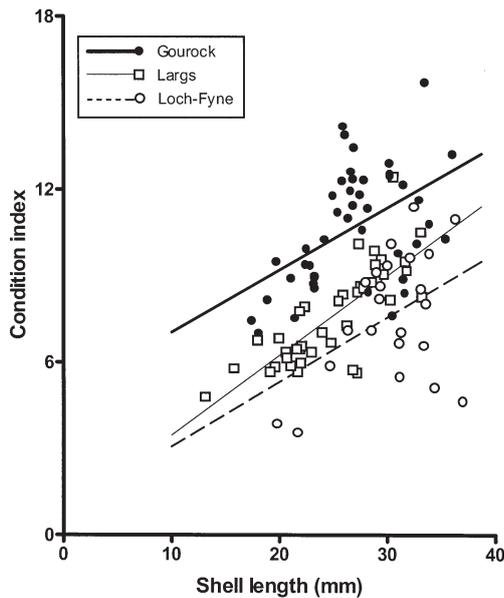


Fig. 3. *Nucella lapillus*. Relationship between shell length and condition index of samples from Gourock, Largs and Loch Fyne

were dependent on size (Fig. 6; Tables 8 & 9). For Cd, the size-dependent relationship varied with population, the Cd concentration in Gourock samples increased in relation to size, but a reverse trend was observed in Largs samples, and Cd concentration in dogwhelks from Loch Fyne was independent of size (slope = 0). For other metals (Cu, Pb and Zn), the concentrations in all samples significantly decreased with increasing size, except Cu and Zn in samples of Gourock and Largs, which were independent of size. At a population level (including different sizes), profiles of metal concentrations of Cd, Cu and Pb in *N. lapillus* were significantly different among areas (Fig. 6; Table 9), but there was no significant difference in Zn between populations. The strong interaction between the concentration versus area and size (Table 9; interaction p < 0.001) indicated that the metal concentration profile in *N. lapillus* not only depended on the effect of the area, but also changed with individual sizes in each population.

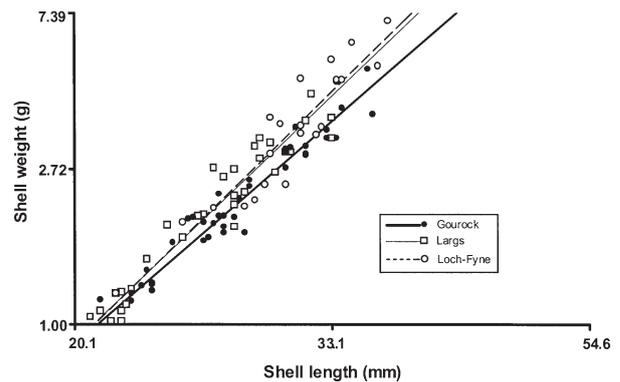


Fig. 4. *Nucella lapillus*. Relationship between shell length and shell weight of samples from Gourock, Largs and Loch Fyne on a double natural logarithmic basis

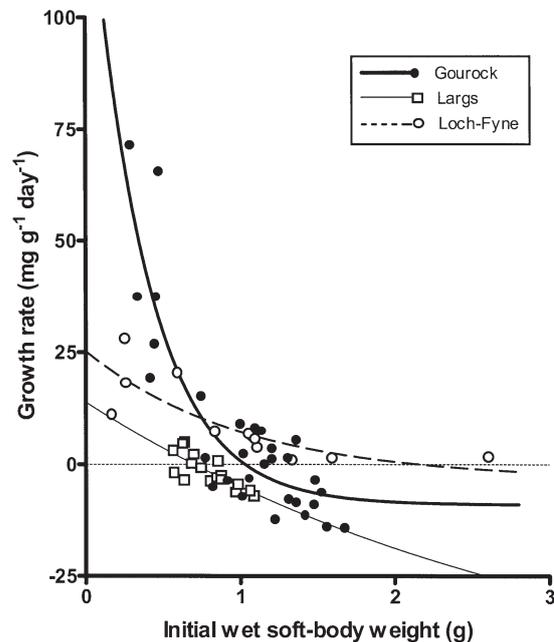


Fig. 5. *Nucella lapillus*. Relationship between initial size and estimated *in situ* growth rate of tagged dogwhelks in Gourock, Largs and Loch Fyne

It had been noted that the growth rate ($\text{mg g}^{-1} \text{d}^{-1}$) of *Nucella lapillus* was very different at initial sizes $<0.7 \text{ g}$ among the populations, with a descending order: Gourrock $>$ Loch Fyne $>$ Largs (Fig. 5). With a view to studying the effect of *in situ* growth rate on metal accumulation, we compared the concentration of metals at an initial size of 0.5 g wet soft-body mass (Fig. 7). The dogwhelks from Largs at this standardised size accumulated significantly higher Cd and Cu in their tissues than the dogwhelks from the other 2 sites (Fig. 7a,b), although there was no significant difference in Pb or Zn concentration among any of the populations. Furthermore, the population of Loch Fyne also had a higher Cd concentration in its tissues than the dogwhelks from Gourrock (Fig. 7a). In accord with the results of Cd and Cu concentrations, the highest MT concentration in the Leiblein gland of *N. lapillus* was observed in the Largs population, while the other 2 populations had similar levels of MT (Fig. 8a; Kruskal-Wallis [KW] statistic = 41.37, $n = 90$, $p < 0.0001$). A

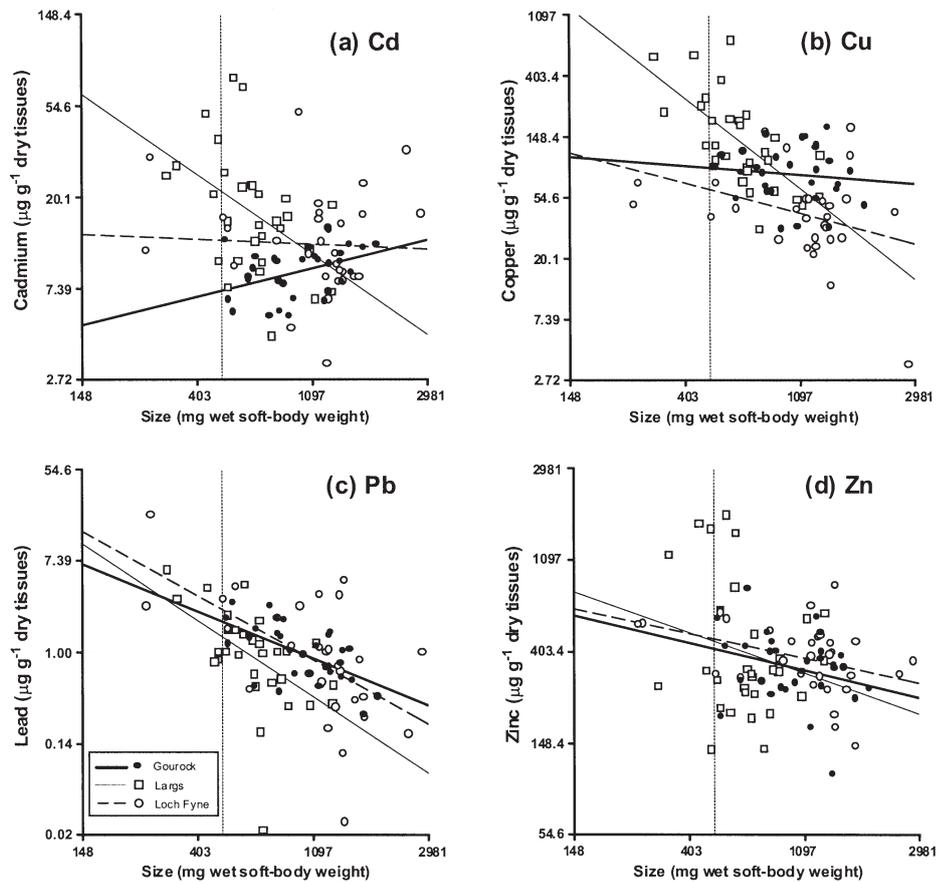


Fig. 6. *Nucella lapillus*. Relationship between size and concentration of Cd, Cu, Pb and Zn in the digestive gland/gonad complex of dogwhelks from Gourrock, Largs and Loch Fyne on a double natural logarithmic basis. Vertical dotted line indicates size at 500 mg wet soft-body weight

Table 8. *Nucella lapillus*. Results of regression analysis between metal concentration in digestive gland/gonad complex and body size ($n = 30$); \ln -concentration ($\mu\text{g g}^{-1}$ wet tissues) is regressed on \ln -dry soft-body weight (mg), where $\ln a$ is the intercept, b is the regression coefficient, and r is the correlation coefficient. *, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

Area	$\ln a$	SE of a	b	SE of b	r	p
Cadmium						
Gourrock	0.051	0.935	+0.310	0.135	+0.397	0.0297*
Largs	8.519	2.095	-0.878	0.322	-0.458	0.0110*
Loch Fyne	2.868	1.358	-0.055	0.194	-0.053	0.7791
Copper						
Gourrock	5.408	1.622	-0.148	0.234	-0.118	0.5336
Largs	14.550	2.025	-1.485	0.312	-0.361	0.0503
Loch Fyne	7.232	1.710	-0.499	0.244	-0.669	<0.0001***
Lead						
Gourrock	7.058	1.810	-1.072	0.262	-0.596	0.0005***
Largs	10.720	3.307	-1.670	0.509	-0.527	0.0028**
Loch Fyne	9.646	2.653	-1.402	0.379	-0.573	0.0009***
Zinc						
Gourrock	7.895	1.372	-0.299	0.198	-0.274	0.1426
Largs	8.873	2.515	-0.444	0.387	-0.212	0.2612
Loch Fyne	7.818	0.866	-0.270	0.124	-0.381	0.0378*

similar result was noted if the MT concentration as expressed per whole Leiblein gland (Fig. 8b; KW statistic = 24.97, $p < 0.0001$). Partial correlation between the concentration of MT-inducing metals (Cd, Cu and Zn) in the digestive gland/gonad complex and MT in the Leiblein gland controlling for size revealed that the MT concentration was very significantly correlated with the concentration of Cd or Cu in *N. lapillus* (for Cd: $r = 0.2932$, $p = 0.005$; for Cu: $r = 0.3103$, $p = 0.003$; all cases with $df = 87$). Unexpectedly, there was a negative correlation between MT and Zn concentrations ($r = -0.2480$, $p = 0.019$).

RNA, the RNA/protein ratio and glycogen in *Nucella lapillus*

Gourrock dogwhelks had a significantly higher RNA concentration in their

Table 9. *Nucella lapillus*. Analyses of covariance of metal concentration in the digestive gland/gonad complex, versus area and size (wet soft-body mass), where size is the covariate. *p < 0.05; **p < 0.01; ***p < 0.001

	df	Cadmium		Copper		Lead		Zinc	
		MS	p	MS	p	MS	p	MS	p
Regression	3	2.093	<0.001***	8.852	<0.001***	10.126	<0.001***	0.668	0.061
Area	2	2.132	0.001**	4.834	<0.001***	3.430	0.016*	0.136	0.596
Size	1	0.347	0.267	6.353	<0.001***	30.162	<0.001***	1.581	<0.016*
Area × size	1	4.231	<0.001***	25.716	<0.001***	29.391	<0.001***	22.419	<0.001***
Residual	86	0.279		0.389		0.795		0.261	

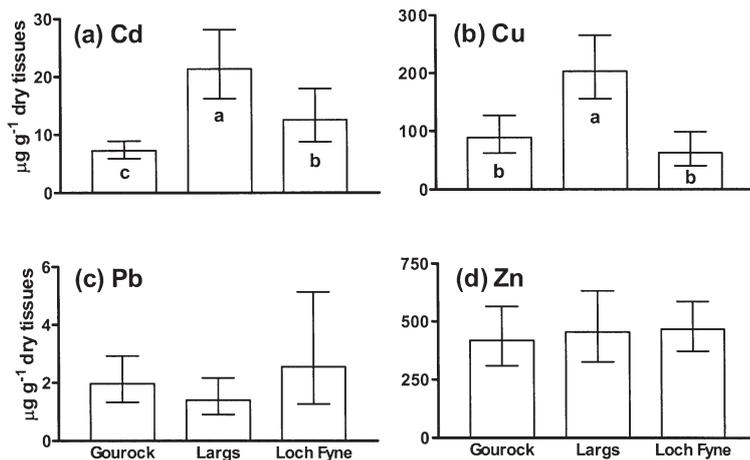


Fig. 7. *Nucella lapillus*. Comparisons of concentration of Cd, Cu, Pb and Zn in the digestive gland/gonad complex of dogwhelks from Gourrock, Largs and Loch Fyne, at a standard size (0.5 g wet soft-body wt)

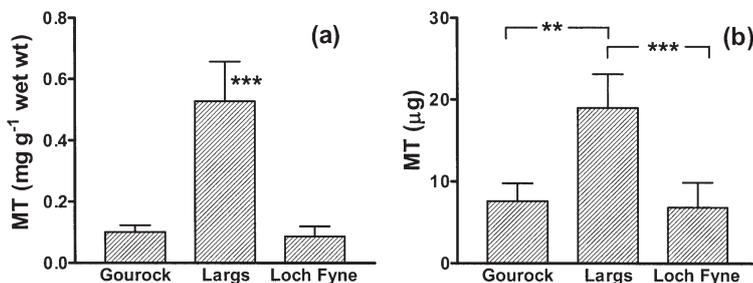


Fig. 8. *Nucella lapillus*. Comparisons between average concentration (a) and content (b) of metallothionein (MT) in the Leiblein gland of dogwhelks from Gourrock, Largs and Loch Fyne. **, ***: significantly different at p < 0.01 and p < 0.001, respectively

foot muscle than individuals from Loch Fyne (Fig. 9a; ANOVA: $F_{2,87} = 5.161$, $p = 0.0076$) and higher RNA/protein ratios than either of the other 2 populations (Fig. 9b; KW statistics = 40.09, $p < 0.0001$). Partial correlation analysis with a correction for size also suggested that the RNA/protein ratio decreased with increasing concentration of MT in the Leiblein gland ($r = -0.3186$, $p = 0.015$, $df = 87$) and with increasing con-

centration of Cd in the digestive gland/gonad complex ($r = -0.3187$, $p = 0.015$).

Glycogen stores varied with the tissues and areas (Fig. 10). In general, the foot muscles stored more glycogen than did the digestive gland. In the foot muscles, the Loch Fyne population showed the lowest glycogen stores (Fig. 10a; ANOVA: $F_{2,87} = 5.529$, $p = 0.0055$). In contrast, the Loch Fyne population had the highest glycogen stores in the digestive gland/gonad complex (Fig. 10b; ANOVA: $F_{2,87} = 6.935$, $p = 0.0016$). These results suggested that there might be differences in energy allocation strategy among different populations.

DISCUSSION

General metal contamination profiles

Based on the concentrations of metals in Chelex and *Mytilus edulis*, Gourrock contained significantly higher concentrations of Pb and Zn than Loch Fyne, while all areas showed similar concentrations of Cd and Cu. Contamination of organotin compounds such as tributyltin (TBT) was also assumed to be greatest in Gourrock, as indicated by the imposex indices and Sn concentration of *Nucella lapillus*. Although *N. lapillus* from Loch Fyne were larger in size (age), they only presented a very low level of imposex and displayed a significantly lower Sn concentration in their digestive glands.

Loch Fyne, therefore, is a cleaner area than the other 2 sites, with less TBT, Pb and Zn in its marine environment, which could be one of the major reasons why the dogwhelk population at this site exhibited a longer life span (as indicated by the size-frequency distribution in Fig. 2).

According to the data obtained from transplanted Chelex and mussels or native mussels, we would ex-

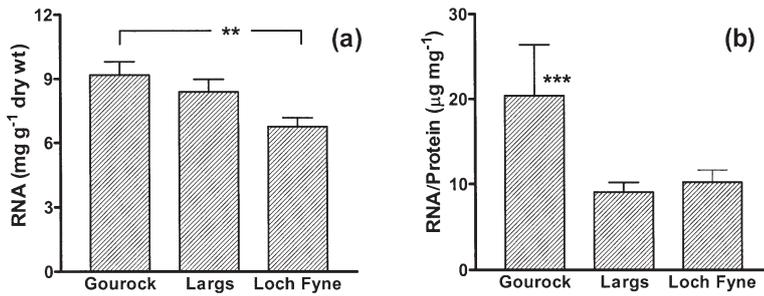


Fig. 9. *Nucella lapillus*. Comparisons between average RNA concentration (a) and the RNA/protein ratio (b) in foot muscle of dogwhelks from Gourock, Largs and Loch Fyne. **, ***: means significantly different at $p < 0.01$ and $p < 0.001$, respectively

pect the *Nucella lapillus* population from Loch Fyne to have lower concentrations of Pb and Zn, and all populations to have similar Cd and Cu concentrations. However, metal contamination profiles generated using *N. lapillus* are very different from these predicted patterns. Although there was no difference in Zn concentration among the populations (Fig. 7d), the concentrations of Zn were much higher than those of the other metals in the dogwhelks. The high concentrations of Zn in *N. lapillus* may come through the food-chain, from barnacles (which accumulated

very high concentrations of Zn: Table 6), as barnacles can continuously accumulate Zn throughout life by storing Zn in phosphate granules (Rainbow et al. 1990, O'Leary & Breen 1997). Comparisons at a standardised size (0.5 g wet soft-body wt) indicated that concentrations of Pb were also similar throughout all sites (Fig. 7c) while dogwhelks from Largs showed significantly higher concentrations of Cd and Cu in their tissues (Fig. 7a,b). Concentrations of Cu in dogwhelks at this size were similar between Gourock and Loch Fyne as predicted, but the latter population showed significantly higher Cd than the former.

As food consumption can be an important route for metal accumulation in marine invertebrates (Wang & Fisher 1999), food consumption rates and metal concentrations in the foods may affect the bioaccumulation of metals by *Nucella lapillus*. Apparently, in the present study, significantly higher Cd and Cu concentrations observed in *N. lapillus* from Largs could be partially attributed to their dominant diet, *Semibalanus balanoides*, which accumulated relatively high concentrations of these 2 elements compared to the native *Mytilus edulis* of the other 2 sites (Table 6). Dogwhelks can effectively regulate and excrete ingested Zn-bound phosphate-granules from the barnacle *S. balanoides* (Nott & Nicolaidou 1990), even though there is a high uptake rate of Zn (because of high concentrations of Zn stored in the phosphate granules of barnacles). These might reasonably explain why there were no significant differences in Zn concentrations of dogwhelks among Largs and the other 2 sites.

Notwithstanding, the influence of dietary metal levels on metal accumulations in *Nucella lapillus* is probably not the sole confounding factor. In both Gourock and Loch Fyne populations, *N. lapillus* could feed on either mussels or barnacles, or both. Further, metal concentrations in native *Mytilus edulis* (Pb and Zn) and *Semibalanus balanoides* (Cu) from Gourock were significantly higher than those from Loch Fyne (Table 6). Based on all the metal data in Chelex, mussels and barnacles (Table 6), Gourock's dogwhelks would be expected to accumulate more Pb, Zn and perhaps Cu from both water and diet than the dogwhelks from Loch Fyne. However, there were no significant differences in Cu, Pb and Zn concentrations of *N. lapillus* between Gourock and Loch Fyne (Fig. 7). Therefore, apart from the effects of dietary metal uptake, other biotic factors may affect the bioaccumulation of metals by *N. lapillus*, such as differences in growth.

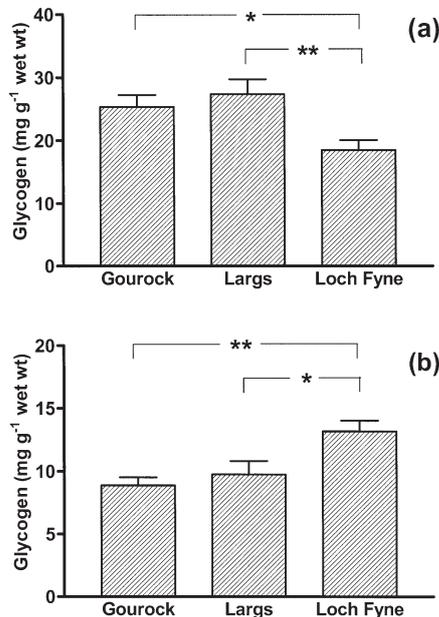


Fig. 10. *Nucella lapillus*. Comparisons between average glycogen concentration in foot muscle (a) and digestive gland/gonad complex (b) of dogwhelks from Gourock, Largs and Loch Fyne. *, **: means significantly different at $p < 0.05$ and $p < 0.01$, respectively

Growth rate confounding *Nucella lapillus* as a biomonitor of metal contamination

Burrows & Hughes (1990) studied the *in situ* growth rates of 2 different populations of *Nucella lapillus*, indicating that individual growth rates decreased with increasing size and varied from -0.3 to 3.2 mg d⁻¹, depending on environmental factors such as shore topography and prey type. In a previous laboratory study, individual growth rates of adult *N. lapillus* varied from -1.6 to 2.5 mg d⁻¹, depending on the nutritional status and prey type (Leung & Furness 2001). In the present field study, we not only observed a consistent negative correlation between growth rate and dogwhelk size, but also a wider variation in the individual growth rate of *N. lapillus* based on these 3 wild populations (Fig. 5). For example, for dogwhelks of 1 g (wet soft-body wt), growth rates ranged from -4.8 to 6.4 mg d⁻¹, and an even wider variation could be observed amongst smaller or younger individuals.

The results of the present *in situ* growth study indicate that the growth rate of small dogwhelks (<0.7 g wet soft-body wt) was highest in Gourock, followed by Loch Fyne and then Largs (Fig. 5). As metal concentration is expressed as $\mu\text{g g}^{-1}$ soft-body weight, dogwhelks with high growth rates may take less time to reach the same body weight than slower growing ones, and thus accumulate less metals. It is therefore likely that this tissue-dilution effect on metal concentrations occurred in *Nucella lapillus* of Gourock, leading to lower concentrations of Cd and Cu and similar concentrations of Pb and Zn compared to the other populations (Fig. 7). In contrast, growth of *N. lapillus* in Largs was significantly slower and the concentrations of Cd and Cu were higher (Fig. 7a,b). Therefore growth rate of *N. lapillus* can primarily explain the differences in the metal-contamination profiles between data generated from Chelex/mussels and *N. lapillus*.

The population from Gourock not only grew well, but also had higher values of CI and presented the highest average RNA or RNA/protein ratios. These parameters have been widely utilised to assess the condition of marine organisms such as scallops (Lodeiros 1996) and oysters (Wright & Hetzel 1985) in the field. In general a higher concentration of RNA or the RNA/protein ratio reflect a better growth, nutritional or health state of an organism (Mayer et al. 1989, Wo et al. 1999), i.e. a better fitness. Better fitness may imply that the animals have a better physiological condition for detoxification and regulation of metals, since combating metal toxicity is metabolically costly (Forbes & Calow 1996). Low metal concentrations in these fast growers might not only be due to the tissue-dilution effect but also to a greater efficiency in detoxification and excretion of trace metals. Overall,

growth rate and health conditions (RNA/protein ratio) are important factors that can confuse metal-contamination profiles when using marine molluscs such as *Nucella lapillus* as biomonitors of trace metals.

Factors influencing growth rate of *Nucella lapillus*

There was no difference in topography, hydrology or the energy level of tidal/wave exposure among the 3 sites—all factors affecting the growth of *Nucella lapillus* (Etter 1996, Kirby et al. 1997). However, we noted that all sites were covered with the barnacle *Semibalanus balanoides*, and/or *Chthamalus* spp., while the mussel *Mytilus edulis* was more frequent in Gourock and Loch Fyne, but not in Largs (Table 2). Feeding on mussels alone or in combination with barnacles promotes better growth in *N. lapillus* than feeding on barnacles alone (Burrows & Hughes 1990, Etter 1996). Thus, the slow growth rate of *N. lapillus* from Largs may be directly linked to their feeding on barnacles, a less profitable prey item.

We also noticed empty shells of *Nucella lapillus* and high numbers of the shore crab *Carcinus maenas* at the sampling site of Largs. Higher predation pressure may restrict the foraging activity of dogwhelks, as there is a trade-off between foraging and hiding within a refuge (Hughes & Burrows 1994, Vadas et al. 1994). It is possible that the dogwhelks in Loch Fyne were also under considerable predation pressure, as suggested by low population density and a high effort per unit catch (the majority of the dogwhelks were hidden under rocks or seaweeds). Palmer (1990) showed that crab effluent and the scent of damaged conspecifics strongly reduces the feeding activity of juvenile dogwhelks, resulting in slower tissue growth and considerable thickening of the shell. In this way, the heavier shells of dogwhelks from Largs and Loch Fyne might also be associated with higher predation pressure.

Any contaminated area, such as Gourock, might be unsuitable for certain predatory species to stay or survive; however the dogwhelk, a species tolerant to contaminants such as metals and TBT, could have grown normally, possibly under a low predation pressure. Pollution-mediated alteration of community structure can be a potentially influential factor changing predation pressure (DeAngelis 1996) that might be favourable to the population of *Nucella lapillus*. As there is a growing concern to integrate studies of environmental pollution, toxicology and ecology, researchers are encouraged to consider beyond the individual organism and to focus on population or community levels when studying toxicity of contaminants (Lagadic et al. 1994, Baird et al. 1996, Calow 1996). Further field experiments including behavioural and ecological studies are

required to confirm whether pollution can enhance the fitness of dogwhelks by lowering predation pressure.

Toxic effects of trace metals on *Nucella lapillus*

Cellular toxicity may result if the rate of metal influx into the cell exceeds the rate of MT synthesis and/or the maximum level of these proteins synthesised by the cell (Viarengo 1985, Di Giulio et al. 1995). Therefore it has been proposed that measurement of MT may provide information about potential health hazards of metals in exposed organisms (Benson et al. 1990, Bebianno & Machado 1997), although MT may be influenced by various biotic and abiotic factors (Mouneyrac et al. 1998, 2000, Leung & Furness 1999a, Leung et al. 2000). The MT concentration observed in the Leiblein gland of *Nucella lapillus* from Largs was 5 times greater than that of the other 2 populations. This implies that the Largs population could be under sublethal stresses caused by the trace metals, especially Cd and Cu, for which MT is a good predictor (based on the results of partial correlation analysis). In addition, MT generally responds to the levels of metals (Ag, Cd, Cu, Hg, Zn) in the tissues which can be affected by growth, as demonstrated in the present study. As the slow-growing *N. lapillus* in Largs accumulated more Cd and Cu, these metals could have induced more MT in their tissues. Thus, growth apparently has an indirect effect on MT induction that also follows the pattern of concentrations of Cd and Cu in this species.

Interestingly, the results of the partial correlation also suggested that either the Cd or MT concentrations correlated negatively with the RNA/protein ratio. This negative correlation might be indicative of a reduction in food consumption at increased metal burdens, although there was no significant correlation between the concentration of each metal and individual growth rate. Previous laboratory studies have demonstrated that low concentrations of Cd or Cu can inhibit normal metabolism and reduce growth through reducing consumption in gastropods (e.g. Lai & Lam 1994, Gomot 1997, Cheung & Wong 1998). In a sub-chronic study, Leung and Furness (2001) observed that the growth rate of Cd-exposed *Nucella lapillus* decreased significantly with increasing Cd concentration in its tissues. The dogwhelks from Largs, at the standardised size (0.5 g wet soft-body wt), had 21.35 mg Cd g⁻¹ and 202.96 mg Cu l⁻¹ in their digestive gland/gonad complex, almost twice the concentrations found in the other populations; this may have further affected growth.

Glycogen represents the readily mobilisable storage form of glucose for most organisms. Lagadic et al.

(1994) proposed using glycogen as a biomarker of environmental pollution because changes in glycogen concentrations are not as transient or sensitive to non-toxicant stress (e.g. temperature and salinity). In laboratory acute studies, waterborne Cd exposure results in reduction of glycogen in *Nucella lapillus* (Abdullah & Ireland 1986, Leung et al. 2000), suggesting that there are costs of combating the toxic effects of Cd. In the present field experiment, the foot muscles stored more glycogen than did the digestive gland/gonad complex, consistent with the laboratory results (Leung & Furness 2001). Higher glycogen stores in the muscles may serve to meet the energy demands for locomotion and mucus secretion, whilst the glycogen in the digestive gland/gonad complex might be reserved for reproductive purposes, as *N. lapillus* can breed throughout the year (Moore 1938a). However, there is no obvious causal relationship between metal-pollution levels and glycogen stores in these wild *N. lapillus* populations. Leung and Furness (2001) also observed that there was no significant effect of Cd on glycogen concentrations in the foot muscles and digestive gland/gonad complex of starved or fed *N. lapillus* following exposure to Cd for 80 d. The present results question the suitability of using glycogen as a stress biomarker in biomonitors, as consumption and reproductive state, apart from the stress caused by chemicals, can influence glycogen stores in *N. lapillus*.

Conclusion

In nature, different populations of *Nucella lapillus* (e.g. Gourock and Largs), exhibited different accumulation of and responses to trace-metal contamination. Much of this variation can be attributed to proximate environmental heterogeneity, including prey composition, predation pressure, hydrology, etc. (Lam 1999). Such variation and uncertainty of toxicity data may be critical for decision-making in risk assessment (e.g. formulating the discharge limit for industrial effluents) based on the results of laboratory toxicity tests with a single strain of a species at each trophic level. As *in situ* field examinations on different populations of a biomonitor species are more ecologically sound and essential to environmental protection and conservation, field and laboratory experiments should be run concurrently and complementary to each other.

It is evident that growth can confound the monitoring results of trace metals using *Nucella lapillus*. In fact, even in the most common biomonitor, *Mytilus edulis*, growth rate and nutritional condition can be very different between areas (Sukhotin & Maximovich 1994, Widdows et al. 1995). Further, data on the growth rate of *M. edulis* and its relationship to size are needed

to model and to predict metal concentration and allometry of metal contamination (Wang & Fisher 1997). Nowadays, the metal concentration of biomonitors of a standardised size is compared between sampling sites, using regression between size (biomass, shell length or shell weight) and metal content (Langston & Spence 1995). However, this method does not resolve the problem of growth-mediated differences in metal concentrations of biomonitors. Although it has been suggested that biomonitoring programmes should use biomonitors of similar size and growth rate, this is not always practical.

Researchers should incorporate measures of *in situ* growth rate and the condition index or the RNA/protein ratio while monitoring trace metals using biomonitors in order to rule out the effect of growth-mediated differences in the metal and MT concentrations. This ecotoxicological biomarker approach is indispensable for monitoring programmes using gastropod molluscs as biomonitors. If resources are limited, any monitoring programme should at least include data on the condition index, which is easy to measure with minimal cost.

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