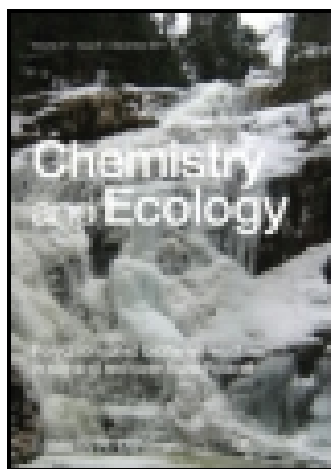


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Spatio-temporal population and reproductive responses in *Perinereis gualpensis* (Polychaeta: Nereididae) from estuaries under different anthropogenic influences

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Spatio-temporal population and reproductive responses in *Perinereis gualpensis* (Polychaeta: Nereididae) from estuaries under different anthropogenic influences

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Reproductive and population-level responses are ecologically relevant diagnostic tools for revealing the cost of long-term tolerance to contaminants. Spatial density distribution, seasonal density, biomass, size frequency histograms and oocytes stages in *Perinereis gualpensis* (Polychaeta: Nereididae) from a highly anthropogenically impacted estuary (Lenga, south-central Chile) were compared with low-polluted estuaries (Tubul and Raqui). Spatial distribution showed that the highest *P. gualpensis* abundances occur in the central (middle) estuarine area, establishing them as suitable zones for comparisons of estuaries. Middle areas also showed differences among estuaries in terms of population and reproductive responses. Mature stage oocytes and juvenile recruitment limited to certain seasons and low adult survival led to significant low densities and biomasses in Lenga individuals ($p < .05$). Conversely, high densities and biomass, continuous recruitment and mature oocyte occurrence were observed in Tubul–Raqui estuaries. These results reflected population and reproductive differences among high- and low-polluted estuaries, suggesting that chronic pollution status in Lenga estuary is a factor to be considered when evaluating these responses.

Keywords: polychaeta; population dynamics; pollution; Mercury; estuary; Chile

1. Introduction

Estuarine environments are exposed to chemical contaminants introduced into the aquatic ecosystems by industrial and domestic activities.[1] The historic and chronic deposition of contaminants results in complex mixtures in estuarine sediments, which, in conjunction with differences in the persistence and bioavailability, makes the assessment of biological effects difficult.[2]

Polychaeta worms of the Nereididae family are one of the most common and representative species of estuarine macrobenthos,[3] and they are classified as key species due to their important role as a food source and in biogeochemistry processes.[1,4] Sustainability of estuarine environments is necessary to maintain the ecological and economic health of coastal regions. So, the assessment of key estuarine population species is ecologically relevant in the environmental assessment of estuarine areas.

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Perinereis gualpensis Jeldes, 1963 (Polychaeta: Nereididae) is a widespread keystone species from southern Chilean estuaries: Lingue River Estuary, Mehuín Bay, southern Chile (39°26'S; 73°13'W) [5]; Queule River Estuary, south-central Chile (39°26'S; 73°13'W) [6,7]; mouth of Cuervo and Condor rivers and Acontilada Bay (45°20'S; 73°05'W).[8] It is an euryhaline species characterised by residing in areas with different degrees of contamination, being a useful biomonitor for polluted sediments.[2,9–11] In terms of reproductive biology, *P. gualpensis* is a semelparous species with sediment fertilisation, eggs protected in gelatinous masses and benthic larval development [12] and reduced mobility which allow determining site-specific responses.

An example of highly anthropogenically impacted system is the Lenga estuary (36°46'15''S; 73°10'06''W), located in San Vicente Bay. It is one of the most industrialised coastal areas of South Eastern Pacific and is historically impacted by mercury, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls and hexachlorobenzene derived from industrial activities around the Lenga estuary.[10,13–15] The long-standing historical events of pollution in Lenga sediments and high persistence of some pollutants, like Hg, in their sediments [10] give an excellent backdrop for understanding the long-term effects of pollution in key estuarine species populations.

Reproductive and population-level responses in nereidids are ecologically relevant diagnostic tools for ecotoxicological studies and for understanding the energetic cost of long-term tolerance to contaminants.[16–18] Since high physiological tolerance to natural stressors such as salinity and temperature is energetically expensive, combating additional stressors derived from human activities might imply an extra energetic cost, diminishing the available resources for growth and reproduction.[19] Population fitness deployment also implies changes in biogeochemical processes such as bioturbation process where nereid polychaetes play an important role.[3] Low densities of bioturbator species could drastically modify sediment reworking/bioirrigation process, inducing changes in the structure of the habitat, affecting finally the local biodiversity.[20]

The study of oocytes' sexual maturity stages throughout the year is a valuable tool for monitoring studies since sex ratio in some estuarine nereid polychaetes greatly favoured females and indicates the time and prevalence of spawning periods during the year.[21,22] Biometric measurements, such as L3, which summarise the length of prostomium, peristomium and first segment, are recommended in animals, which can easily lose part of their body.[23,24] L3 size frequency histograms are a valuable tool to determine the number of cohorts and recruitment patterns in polychaetes.[17,23,25] Ragworms' population densities and biomasses also represent classic ecological endpoints to compare populations and secondary production between different estuarine areas.[26] A downward trend in densities and biomass was observed in polluted estuaries compared with reference areas with low anthropogenic pressure.[26]

The aims of this study were to determine spatio-temporal population (L3 length frequency histograms, density and biomass) and reproductive responses (oocytes stages) in *P. gualpensis* from historically polluted sites (Lenga estuary) compared with low-polluted estuaries. Reproductive and population responses allow understanding the main differences in population fitness of *P. gualpensis* among polluted and low-polluted estuarine sediments.

2. Materials and methods

2.1. Study area and sampling sites

Lenga, Tubul and Raqui estuaries are located in south-central Chile (39°S, 73°W, Figure 1). Lenga is a small estuary (3.2 km²) located in San Vicente Bay, Biobío Region, Chile (36°45' S; 73°10' W), impacted by an adjacent chemical industrial complex including an oil refinery, steel mill and chemical industries. Tubul and Raqui estuaries (37°14'S; 73°26'W; Figure 1) correspond

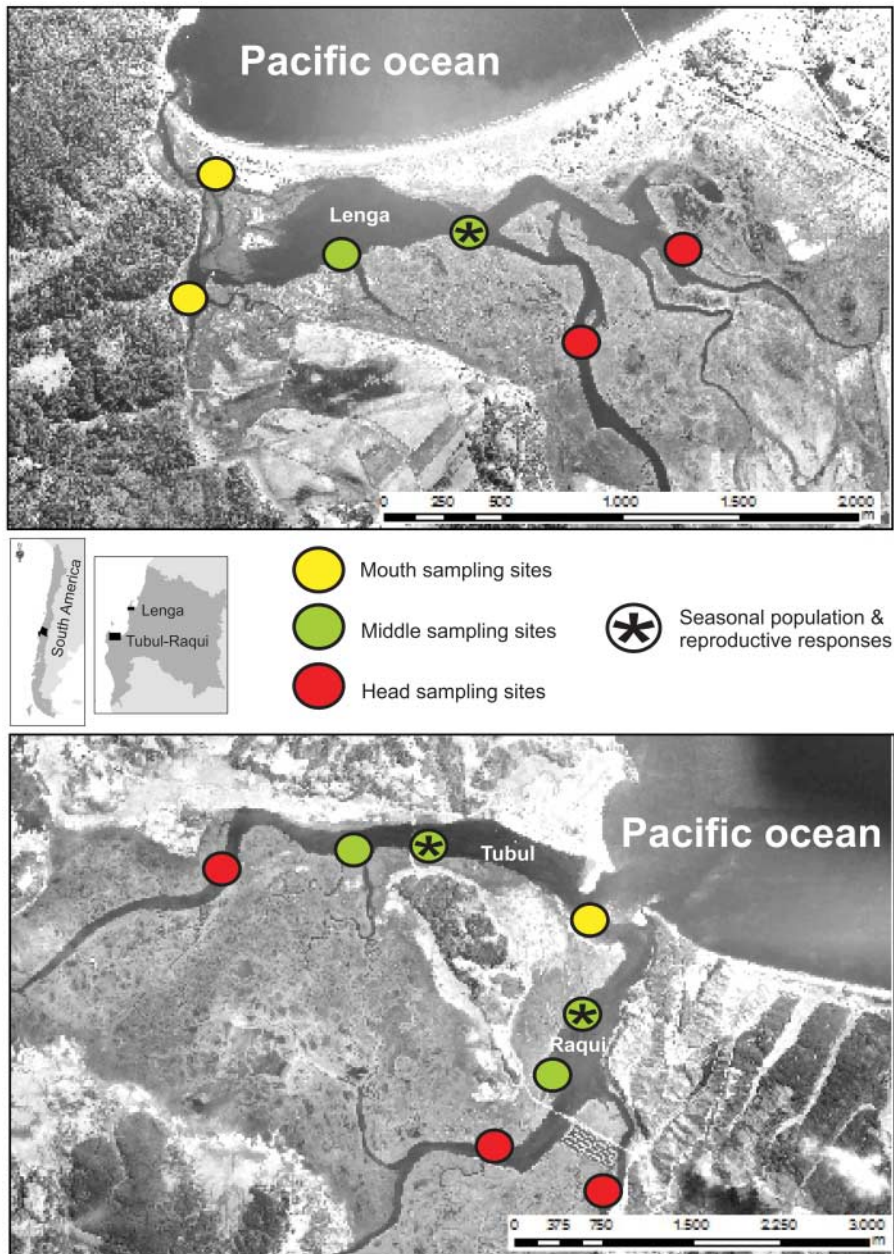


Figure 1. *P. gualpensis* spatio-temporal sampling sites from Lenga and Tubul-Raqui estuaries in south-central Chile.

with two estuaries located in a small coastal-type basin. The low anthropogenic influence and the relative similarity and proximity to the Lenga estuary make it an appropriate reference site for comparison purposes.[2] Moreover, considering that those estuarine basins receive freshwater inputs only in the rainy season,[2] similar hydrological patterns in terms of seasonal salinity changes are ensured.

Spatial trends of *P. gualpensis* abundances were determined by sampling six and eight sites in Lenga and Tubul-Raqui estuaries, respectively (Figure 1). Sampling sites were grouped in head, middle and mouth estuarine zones to determine the wide range of *P. gualpensis* abundances

in each estuary (Figure 1). For temporal population and reproductive responses, one site from the middle zone was sampled in each estuary and selected considering *P. gualpensis* densities (> 1000 individuals/m²; Figure 1). Seasonal sampling was performed during representative months: winter (August 2008), spring (November 2008), summer (January 2009) and autumn (April 2009).

2.2. Sampling procedure

Samples were collected in the intertidal zone at low tide. Population and reproductive responses were assessed based on two (spatial) and four (temporal) replicate samples. Ragworms were collected at each station using a PVC core (30 cm \times 12 cm), and replicates were collected at a distance of five metres from each other. Each sample was sieved to 0.5 mm, and the sampled organisms were fixed in 10% seawater–formalin and subsequently preserved in 70% ethanol until analysis. In order to characterise the sediment's physicochemical properties, samples were collected in winter and summer by using acrylic cores ($n = 2$, per sampling site). Sediments samples were kept at -20°C until analysis.[2] Redox sediment condition was measured *in situ* from surface sediment layers (0–5 cm). At the same time (or moment) to sediment sampling, *in situ* seasonal measurements of physicochemical properties of water (salinity, temperature, pH and oxygen concentration) were made during low tide, using a multiparameter probe.

2.3. Sediment physicochemical analysis

Sediment physicochemical variables, mean grain size (ϕ), sorting (ϕ), organic matter content (%) and Redox (mV) were analysed according to Díaz-Jaramillo et al. [2] Total Hg and PAHs levels from Lenga, Tubul and Raqui estuaries were mainly gathered from recent and historic data (Table 1).[2,13,15] Total PAHs in sediments were analysed by gas chromatography coupled to a mass spectrometer (GC, Agilent 7890A coupled with MS Agilent 5975 detector). Each sample was quantified for 3–6-ring PAHs for Environmental Protection Agency's 16 priority PAHs (without naphthalene) plus benzo[e]pyrene, perylene 3-methylphenanthrene, 2-methylphenanthrene, 4H-ciclopenta(def)phenanthrene, 4-methylphenanthrene and 1-methylphenanthrene. Reference material and external standards were analysed as reported in [2].

2.4. *P. gualpensis* population and reproductive responses

2.4.1. Density and biomass

Density measurement of nereid polychaetes was performed by counting all individuals sorted by stereomicroscope. Mean seasonal biomass was measured as ash-free dry weight of *P. gualpensis* individuals following the standard procedures.

2.4.2. Size frequency histograms (L3 length)

For L3 measurement, fixed individuals were counted and measured with a micrometer on a stereomicroscope. The L3 size frequency histograms were made using FISAT II free software and treated by Modal Progression Analysis (Battacharya's Method) to determinate potential cohorts.[27]

Table 1. Water and sediment physicochemical variables (Mean \pm S.D) from different estuarine areas of Lenga (L), Tubul (T) and Raqui (R) estuaries.

Water/sediment variable	Estuary	Mouth	Middle	Head	Source
<i>Water</i>					
Salinity (PSU)	L	26.98 \pm 5.20	19.03 \pm 10.87	10.23 \pm 4.26	Present work
	T	21.27 \pm 12.60	17.84 \pm 13.45	16.10 \pm 21.70	Present work
	R	21.27 \pm 12.60	16.21 \pm 11.04	12.95 \pm 11.85	Present work
Oxygen (mg/L)	L	9.47 \pm 1.27	10.20 \pm 2.46	6.70 \pm 2.08	Present work
	T	8.85 \pm 0.07	9.50 \pm 0.75	9.55 \pm 0.07	Present work
	R	8.85 \pm 0.07	9.90 \pm 0.61	9.35 \pm 0.64	Present work
pH	L	8.47 \pm 0.21	7.63 \pm 0.58	7.13 \pm 0.13	Present work
	T	7.80 \pm 0.57	7.74 \pm 0.58	7.30 \pm 0.42	Present work
	R	7.80 \pm 0.57	7.72 \pm 0.68	7.15 \pm 0.17	Present work
Temperature ($^{\circ}$ C)	L	14.59 \pm 3.34	15.13 \pm 6.90	12.20 \pm 7.10	Present work
	T	16.15 \pm 12.66	15.54 \pm 2.16	13.80 \pm 1.27	Present work
	R	16.15 \pm 12.66	17.75 \pm 9.62	14.13 \pm 1.03	Present work
<i>Sediment</i>					
Mean grain size (Phi)	L	2.70 \pm 0.82	2.59 \pm 0.09	5.50 \pm 0.73	Present work
	T	1.22 \pm 0.03	2.26 \pm 0.32	2.96 \pm 0.07	Present work
	R	1.22 \pm 0.03	2.09 \pm 0.17	1.92 \pm 0.38	Present work
Sediment class.	L	Fine sand	Fine sand	Silt	Present work
	T	Medium sand	Fine sand	Fine sand	Present work
	R	Medium sand	Fine sand	Medium sand	Present work
Organic matter (%)	L	6.49 \pm 4.14	2.30 \pm 0.45	22.83 \pm 4.37	Present work
	T	1.45 \pm 0.07	1.67 \pm 0.32	3.35 \pm 0.78	Present work
	R	1.45 \pm 0.07	1.96 \pm 0.55	3.58 \pm 0.61	Present work
Redox (mv)	L	-140.24 \pm 20.59	-127.43 \pm 32.28	-297.43 \pm 69.83	Present work
	T	-102.50 \pm 10.61	-107.31 \pm 67.55	-204.00 \pm 9.43	Present work
	R	-102.50 \pm 10.61	-140.09 \pm 39.59	-172.50 \pm 27.89	Present work
Mercury (mg/kg)	L	0.48 \pm 0.10	4.48 \pm 5.59	30.50 \pm 25.96	[2,15]
	T	N/A	0.02 \pm 0.01	N/A	[2]
	R	N/A	$7 \times 10^{-3} \pm 2 \times 10^{-3}$	N/A	[2]
Total PAHs (ng/g)	L	943.01 \pm 609.87	282.90 \pm 248.17	298.60 \pm 87.38	Present work, [2,13]
	T	N/A	36.99 \pm 39.65	N/A	[2]
	R	N/A	21.57 \pm 14.81	N/A	[2]

2.4.3. Oocyte sexual maturity stages

Female worms (up to 1.30 mm L3 length) obtained from sediment cores were examined in each site from all seasons. In order to determine oocyte stages, an incision of female individuals using a scalpel, approximately 20–23 segments behind the head, was performed. The content of three segments was deposited on a glass slide, and the coelomic fluid was examined under microscope. Four sexual maturity stages were defined in female worms: worms at stage I correspond to sexually undifferentiated specimens (absence of the germ cells); at stage II (development stage), content of some primary oocytes in germinal cell mass was observed (oocyte diameter ranges from 25 to 80 μ m); at stage III (growth stage), growing oocytes (80–190 μ m) were found to be freely suspended in the coelomic fluid and at stage IV (mature stage) oocytes were large, spherical and ready to be spawned (> 200 μ m). No degenerative or scarce spherical oocyte stages were included.

2.4.4. Statistical analysis

The changes in population density and biomass responses were evaluated by analysis of variance with Tukey' test for *post hoc* comparisons ($p < .05$), in order to compare the means obtained at different sites in all seasons. Previously, the assumptions of normality and homogeneity of

variances were verified. The significance of *P. gualpensis* oocyte stages between estuaries at different seasons was evaluated by contingency tables using the chi-square test ($p < .05$) of categorical frequency data.

3. Results

3.1. Water and sediment physicochemical variables

Water physicochemical variables showed a similar pattern among estuaries in terms of salinity, temperature, pH and dissolved oxygen (Table 1). On this basis, the middle estuarine zones showed similar physicochemical characteristics of water among estuaries as well as in their sediments (Table 1). Regardless of similar physicochemical characteristics of sediment and water among estuaries in the middle area, Hg and PAH levels from Lenga showed 2-fold and 1-fold changes, these being higher compared with Tubul and Raqui estuaries (Table 1). Hg levels in Lenga were higher in the head area; conversely, higher total PAHs levels were reported in the mouth estuarine zone (Table 1).

3.2. *P. gualpensis* population and reproductive responses

3.2.1. Density and biomass

Spatial *P. gualpensis* abundance exhibited an inverted *U*-shaped pattern of distribution in all estuaries where middle estuarine zones reflected the highest ragworm densities (Figure 2). Furthermore, Raqui abundances showed significant higher densities compared with Lenga middle area ($p < .05$; Figure 2). Individuals per m^2 from Tubul and Raqui estuaries showed no significant differences between seasons and sites ($p < .05$; Figure 3(a)). Population densities from Lenga showed significant differences between seasons ($p < .05$), with a significant increase in spring densities in contrast to summer (Figure 3(a)). Inter-site differences in population densities were mainly related to the significant differences of Lenga respect Raqui estuary in winter and summer, respectively ($p < .05$; Figure 3(a)).

The biomass of *P. gualpensis* showed no significant difference between seasons in all sampling sites ($p < .05$; Figure 3(b)). Inter-site differences in *P. gualpensis* biomass were related to the significant decrease of grams per m^2 as from Lenga compared to Tubul and Raqui estuaries ($p < .05$; Figure 3(b)).



Figure 2. Spatial density (Mean \pm S.D) in *P. gualpensis* sampling sites from different estuarine areas in Lenga, Tubul and Raqui estuaries. Different letters indicate significant differences among estuarine middle areas ($p < 0.05$).

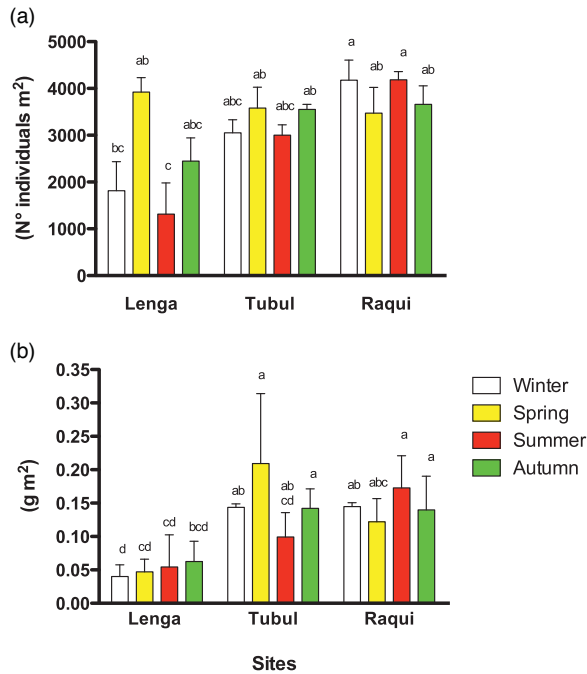


Figure 3. Seasonal density (a) and Biomass (b) (Mean \pm S.D) in *P. gualpensis* sampling sites from Lenga and Tubul-Raqui estuaries. Winter and summer data were obtained from a previous biomarker study.[2] Different letters indicate significant differences among sampling sites and seasons ($p < 0.05$).

3.2.2. Size frequency histograms

The size frequency distribution of *P. gualpensis* from Lenga and Tubul–Raqui sampling sites using L3 length is shown in Figure 4. Size frequency histograms were separated into five size classes from 0.25 to 2.25 mm. Most of the size frequency histograms from all estuarine sites were unimodal. Lenga histograms showed a small number of adult size individuals of 1.25–2.25 mm between winter and spring and recruitment period preferably in these seasons (Figure 4). Moreover, Lenga showed a high percentage of adult size individuals (> 1.25 mm) above 50% in summer and autumn seasons (Figure 4). Two cohorts of young and old individuals were found during winter in Tubul estuary, while no plurimodal size frequency histograms were observed in the remaining seasons (Figure 4). Also, a similar proportion of young and adult size individuals between seasons slightly in favour of young individuals was reported for Tubul estuary. Population from Raqui estuary showed a similar pattern to that of Tubul populations with high proportion of young and adult sizes in all seasons (Figure 4). Inter-site comparisons showed differences between Lenga and Tubul–Raqui *P. gualpensis* populations, which are generally related to a high number of young and adult individuals and a consistent seasonal pattern of recruitment from Tubul and Raqui estuaries compared with those from Lenga.

3.2.3. Oocyte sexual maturity stages

P. gualpensis oocyte sexual maturity stage is shown in Figure 5. Sexually undifferentiated worms (stage I) and oocytes in development stage (stage II) were observed in all sampling sites and seasons (Figure 5). Growth (stage III) and mature (stage IV) oocyte stages from Tubul and Raqui were observed in all seasons and only in Raqui site in spring, stage IV was absent (Figure 5).

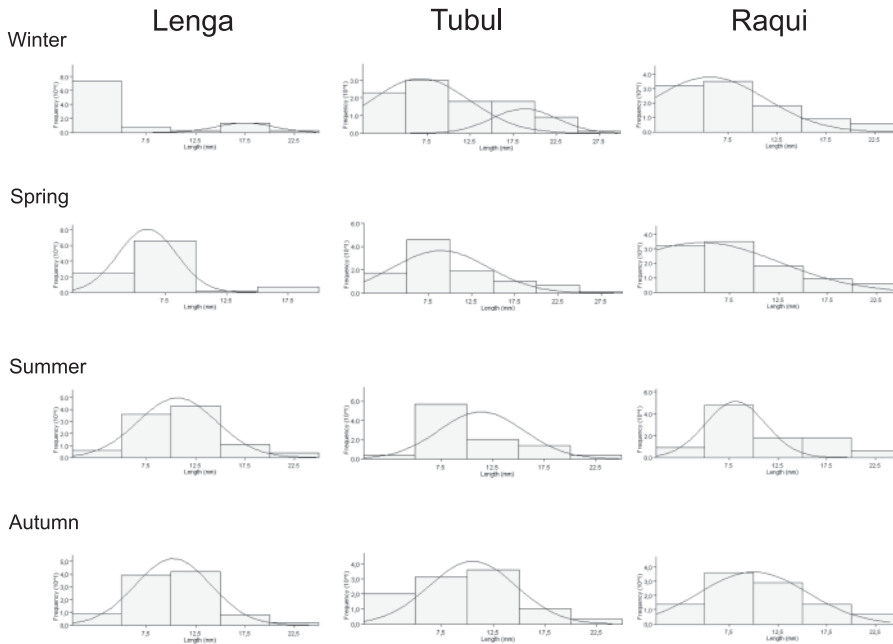


Figure 4. Seasonal size frequency histograms (L3 in mm) in *P. gualpensis* collected from Lenga, Tubul and Raqui estuaries. Winter and summer data were obtained from a previous biomarker study.[2]

Mature and growth stages from Lenga appeared only in certain seasons. Growth-stage oocytes from Lenga were present only in winter, spring and autumn, and mature oocytes were present only in winter, being absent in the remaining seasons (Figure 5). Oocyte stages percentage from Lenga ragworms were different compared to Tubul and Raqui ragworms in all seasons ($p < .05$). Inter-site differences between Lenga and Tubul–Raqui estuaries were mainly related to the active gametogenesis (growth and mature stages) observed in Tubul and Raqui estuaries for all the seasons compared with females from Lenga estuary (Figure 5).

4. Discussion

Sampling sites were similar in terms of water and sediment physicochemical parameters, and these values are similar to those reported in other estuaries of south-central Chile.[9] Middle estuarine areas exhibited higher similarities in terms of water and sediment characteristics and emerged as the most suitable areas for inter-estuarine comparisons. Similarity in key forcing factors such as salinity, temperature and sediment quality among the majority of studied sites ensures proper comparison. These factors help to shape the nature of benthic communities and the key functional processes in coastal seas and estuaries.[16] Furthermore, *P. gualpensis* showed a *U*-shaped distribution pattern typically found in estuarine animals.[28] It ensures the middle areas as proper sites for inter-estuarine comparisons according to the highest densities and the best scenario for the population development within an estuary.

High levels of environmental pollutants detected in Lenga sediments, in many cases with persistent characteristics and above some Sediment Quality Guidelines [2,10,11,13–15], make it possible to classify it as a chronic and heavily polluted estuary. Hg distribution pattern in Lenga sediments (> head > middle > mouth) suggests that concentrations depend on the distance from the source rather than on sediment's physicochemical characteristics.[10] On the other hand,

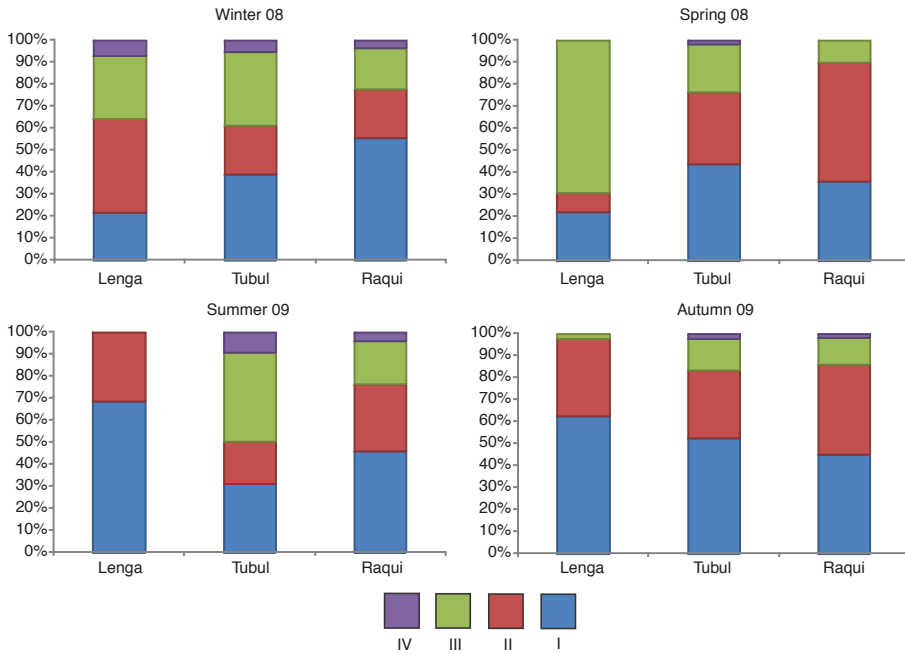


Figure 5. Seasonal sexual maturation percentage of the four sexual maturity stages (I–IV) (oocyte maturation stages proportion) in *P. gualpensis* collected from Lenga, Tubul and Raqui estuaries.

high levels of Hg and their organic forms reported in *P. gualpensis* tissues from similar sampling sites in Lenga [10,11] demonstrate that part of Hg in their sediments is bioavailable to ragworm populations. PAHs distribution pattern in Lenga sediments (> mouth > middle > head) suggests the influence of recent oil spillage events from the adjacent bay in PAHs sediment deposition close to the mouth area.[2] Finally, in terms of those contaminants, Tubul and Raqui sites have been classified as low-impacted estuaries, which would be related to the absence of large-scale industrial activity around these coastal basins.[2,10,11]

Population responses from Lenga individuals are related to low-density biomass and strong seasonal changes in the number of individuals per m^2 . The high number of individuals in Lenga spring similar to those found in reference and other south central estuaries (up to 2000 individuals/ m^2) [7] first suggests that *P. gualpensis* populations from Lenga could reach densities similar to non-/low-impacted estuaries. On the other hand, it also indicates that winter and spring seasons represent the main recruitment periods, when a great number of juvenile individuals are incorporated into the population. However, a significant decrease in the next seasons clearly indicates an effect of adult survival, which is corroborated with low biomass per m^2 and small proportion of larger size classes in Lenga ragworms. Low densities and biomass first imply reduced food transference to higher trophic levels.[29] indicating a potential depletion of energy fluxes from Lenga estuary.

Breeding period differences among studied estuaries are according to seasonal L3 length frequency histograms in terms of juvenile recruitment inferred by the small size length class. Ragworm populations from Tubul and Raqui estuaries showed a constant high proportion of juveniles in all seasons, in some cases being higher than 80% in contrast to Lenga, where ‘maximum peak’ was observed during winter to spring. Size frequency histograms in Lenga and Tubul-Raqui sites showed mainly unimodal cohorts in agreement with other nereid species, [29–31] but different from other related species with plurimodal cohorts.[23,25] Inter-estuary differences were mainly due to more widespread cohorts with higher adult survival in Tubul and

Raqui, and a strong decrease in adult sizes in Lenga. Callow et al. [32] suggest that the effects of toxicants in semelparous species are mainly focused on reduction of juvenile survival or fecundity compared with iteroparous species. These findings suggest that the decreases of adult polychaetes in Lenga might be the consequence of the exposure to highly polluted sediments, leading to a low number of adult survivors.

Similar oocyte stages proportion and the occurrence of mature oocytes in all seasons from low and non-impacted estuaries indicated an active gametogenesis during the year similar to that reported in clean estuarine areas.[17] In contrast, Lenga ragworms showed mature oocyte stages only in certain seasons, indicating that breeding seasons from Lenga are focused in short periods compared to non-impacted populations.

Delayed reproductive maturation in polychaetes has been shown in response to contaminant exposure.[33] The bioenergetics approach related to energetic cost of tolerance to contaminants could also be a reasonable explanation.[19,22] The reallocation of energy results in individual responses such as changes in time to reproduction, or reproductive success.[34] However, Hg, as other pollutants from oil industry (crude oil derivatives and phenol-based compounds), acts as endocrine disruptor chemicals (EDCs) in invertebrates.[35] Thus, further studies should focus on a new sediment characterisation based on some EDCs compounds in order to assess the contribution of other oil industry chemicals in pollution problems from Lenga. Also, the development of Vitellogenin (Vg) and Energy Metabolism biomarkers is an endpoint that should be assessed in the future, [22,35] helping to determine which mechanisms are involved in reproductive differences observed in Lenga ragworms.

The assessment of pollution-related responses by population-level endpoints is difficult.[36] A significant body of literature highlights that this spatial and temporal heterogeneity cannot be related only to pollution levels.[16,37] Also, genetic isolation would add a further layer of population variability among estuaries.[38] However, it has been suggested that contaminants might promote microevolutionary processes, such as genetic drift and selection in some nereid populations.[38,39] Differences in spawning periods in some nereid species between geographically distant populations were reported and related to differences in temperature and photoperiod.[3,22] The proximity among these coastal type basin estuaries dismisses the influence of the above-mentioned factors. Nevertheless, the complexity of establishing pollution-related responses by the currently used responses, differential exposure and damaged pollution biomarkers observed in *P. gualpensis* from Lenga sediments [2] represent lines of evidence for related population deployment by high levels of concerning pollutants.

4.1. Conclusions

Differences among estuaries were evident in terms of *P. gualpensis* population and reproductive responses. Mature oocytes and juvenile recruitment were restricted to certain seasons and low adult survival, influencing depleted densities and biomasses from impacted estuary. On this basis, oocyte stages, L3 length frequency, density and biomass on nereid polychaetes appear to be non-expensive endpoints to evaluate ecological outcomes in estuarine areas. However, it is important to take into consideration the exposure time to pollutants since these responses were found in individuals chronically exposed to polluted sediments.

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Disclosure statement

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