

Over-exploitation of *Pyura chilensis* (Asciidiacea) in southern Chile: the urgent need to establish marine reserves

Sobre-explotación de *Pyura chilensis* (Asciidiacea) en el sur de Chile: la urgente necesidad de establecer más reservas marinas

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ABSTRACT

I use data on the intertidal abundance and size frequency of one of Chile's commercially exploited invertebrate species, the "Piure" (*Pyura chilensis*) to argue that intertidal populations of this species are dramatically over-exploited. Here, I compare a population at the Mehuin Marine Reserve with four exploited sites. Densities of *P. chilensis* were more than 3 orders of magnitude higher in the Marine Reserve compared to these sites. Within the reserve, I recorded an average density of $576 \pm 83 \text{ m}^{-2}$ ($\pm \text{sem}$, median=400), an average size (wet weight) $15.3 \pm 1.5 \text{ g}$ ($\pm \text{sem}$) and a maximum size of 112 g. Outside of the reserve, individuals rarely achieved a size of more than 20 g. As *P. chilensis* became reproductive at between 12 and 13 g, this corresponds to only around 6% of individuals in the harvested populations achieving sexual maturity and compares with an average value of $37 \pm 4.8\%$ ($\pm \text{sem}$) within the reserve. A snapshot examination of patterns of recruitment within the reserve, revealed that juveniles only recruited successfully in the presence of adult conspecifics, indicating that recovery of exploited populations is likely to be slow. This study represents one of a number in Chile that underscore the dramatic effects of human harvesting on rocky intertidal communities. I urge Chilean authorities to heed the warnings of their marine scientists, as there is an urgent need to fund the establishment of protected marine areas.

Key words: ascidian, density, harvesting, size-frequency, recruitment

RESUMEN

Utilizando datos de abundancia y frecuencia de tallas del invertebrado *Pyura chilensis* ("Piure"), obtenidos durante este estudio comparativo de las poblaciones de la Reserva Marina de Mehuin y poblaciones de localidades vecinas explotadas, se puede argumentar que las poblaciones intermareales de esta especie están dramáticamente explotadas. Las densidades de *P. chilensis* en la Reserva fueron 3 órdenes de magnitud mayores que en los sitios explotados. En la Reserva registré una densidad promedio de $576 \pm 83 \text{ m}^{-2}$ ($\pm \text{sem}$), un peso húmedo promedio de $15,3 \pm 1,5 \text{ g}$ ($\pm \text{sem}$, mediana=400), y un peso máximo de 112 g. En tanto que fuera de la Reserva los individuos rara vez presentaron un peso mayor a 20 g. La talla de madurez sexual (12-13 g) la alcanzó sólo el 6% de los individuos de las poblaciones explotadas, en cambio la proporción de individuos reproductivos dentro de la Reserva fue de $37\% (\pm 4,8\%)$. Un rápido examen de los patrones de reclutamiento en la Reserva, reveló que los juveniles reclutaron exitosamente sólo en presencia de individuos conspecíficos, lo que estaría indicando una baja recuperación de las poblaciones de esta especie en los sitios explotados. Este estudio es uno más de los varios realizados en comunidades intermareales rocosas chilenas que muestran el efecto dramático de la intervención humana. Debido a ello, hago un llamado a las autoridades chilenas a que presten atención a la urgente necesidad de establecer nuevas áreas marinas protegidas.

Palabras clave: ascidia, densidad, explotación, frecuencia de tallas, reclutamiento

INTRODUCTION

Temperate marine ecosystems are renowned for their diversity and productivity (Suchanek 1994). In Chile, the striking diversity and biomass of organisms on the rocky coast is matched by the degree to which these resources are exploited. After a visit to Chile in 1974, R.T. Paine noted that ... "I believe that the Chilean shoreline

is unique among temperate zone rocky shores in the high level of human exploitation to which it is subject. This is perhaps even more surprising when one considers the coasts generally exposed nature, rough water and few harbours." (cited in Castilla 1976). Among the species that Paine considered to have undergone "...local reductions in abundance, and significant changes in distribution..." he

lists both species of intertidal ascidian; *Pyura chilensis* Molina 1782 and *P. praeputialis* (Heller 1878)¹.

More than 11 genera of marine invertebrates and 7 genera of intertidal algal are exploited on the coast of Chile and frequently a number of species within each genus are harvested (Castilla 1976). The high level of exploitation of these organisms and the ensuing changes in the structure and dynamics of intertidal communities when humans are excluded, is truly dramatic (Moreno *et al.* 1984, 1986, Durán *et al.* 1987, Durán & Castilla 1989, Godoy & Moreno 1989,²).

Here I focus attention on the commercially exploited ascidian *Pyura chilensis*. This species commonly occurs in the low intertidal and subtidal zone (depth to 70 m) from the Island of Chiloé (Golfo de Ancud - 42°S) to southern Perú (Van Name 1945, Brattström & Johanssen 1983). Intertidal and subtidal populations of this animal are commercially exploited (CA Moreno, personal communication). Here, I restrict

myself to the study of intertidal populations. The effects of human exploitation have not been examined for this species (but see footnote 2) and previous studies of *P. chilensis* have focussed largely on individual growth and reproductive biology of populations on Chile's northern coast (Gutiérrez & Lay 1965, Cea 1973). I am not aware of any current data on distribution and abundance of this species. Indeed Santelices *et al.* (1977) in a study which they described as "the first quantitative description of intertidal communities along the Pacific South American Coast" make no mention of *Pyura chilensis*. To what extent this may reflect over-exploitation of this species is not clear. Other references to the distribution and abundance of *P. chilensis* appear to have, on occasions,

¹ It is noteworthy that this later species is referred to as *Pyura bradleyi* by Van Name in his treatise on North and South American Ascidiaceans (Van Name 1945).

² Moreno CA (1994) Ecología de *Choromytilus chorus* en un ambiente intermareal: un estudio de largo plazo. p. 99. Resúmenes, XIV Jornadas de Ciencias del Mar, Puerto Montt.

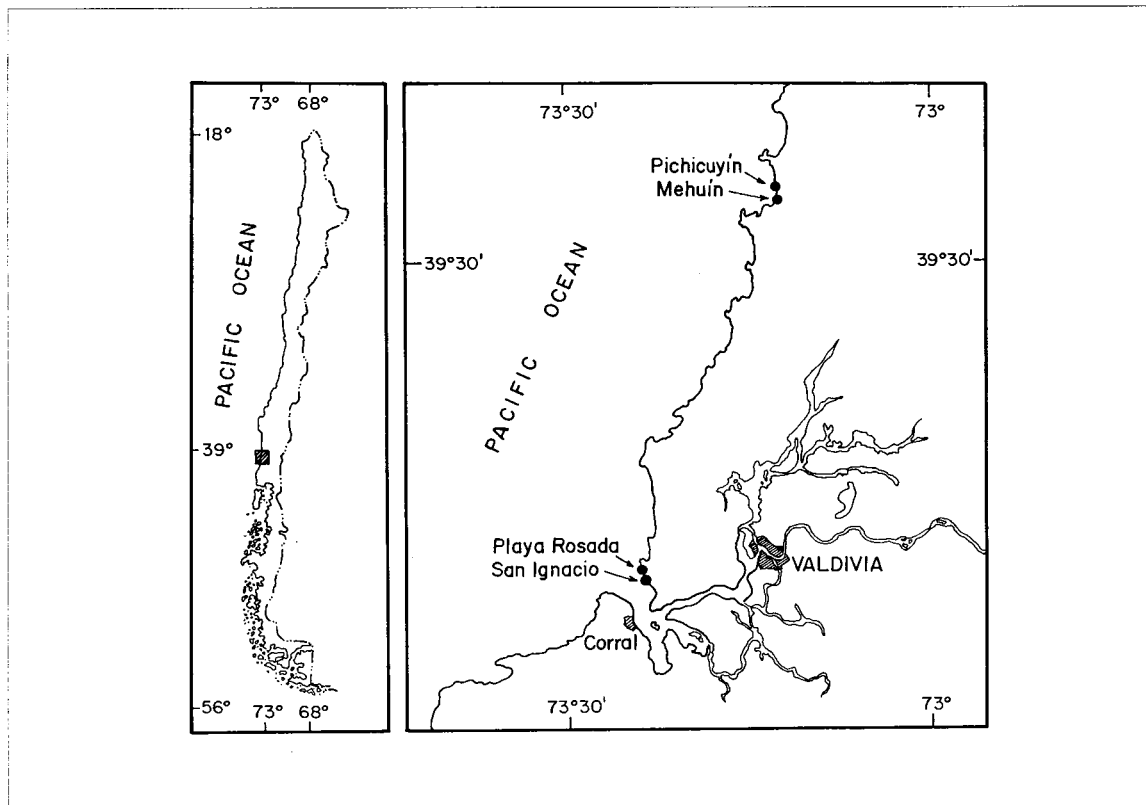


Fig. 1: Location of the four study sites in southern Chile. Mehuín is the only non-harvested site.

Ubicación geográfica de los cuatro sitios de estudio en el sur de Chile. Solamente Mehuín tiene una Reserva Marina.

confused this species with *Pyura praeputialis* (Santelices 1989). This pyurid is also found in the rocky intertidal zone, but has a distinctive upright morphology and is restricted to rocky shores in the vicinity of Antofagasta, northern Chile (Paine & Suchanek, 1983).

Here I examine intertidal populations of *P. chilensis* at three harvested sites and within a Marine Reserve. Specifically, I compare densities and size frequencies at harvested sites with those in the Reserve. I also examine patterns of recruitment in *P. chilensis* within the Marine Reserve.

MATERIALS AND METHODS

Study sites

The density and size frequency of *P. chilensis* was examined at four sites. The Marine Reserve at Mehuin (39° 24' S; 73° 13' W) was closed to the general public and was the only non-harvested site. Monthly size frequency data was collected at this site in January through April, 1994. The three sites from which organisms were harvested lie to the north and south of Mehuin and were selected because of their similarity in wave exposure and mix of species to the Marine Reserve (Fig. 1). Pichicuyin is the next headland directly north of the Marine Reserve and is separated from the Reserve by approximately 1 km of sandy beach. Two adjoining headlands comprise the sites to the south; San Ignacio (39° 50' S; 73° 24' W) is separated from the more northerly headland at Playa Rosada by approximately 2 km of sandy beach. All harvested sites were sampled in March, 1994.

Determining the best predictor of biomass

Pyura chilensis is often irregular in shape and it seemed that the small papillae that project from the surface of the test might render estimates of size based on linear measurements inaccurate. In mid-January 1994, a sample of 46 individuals of *P. chilensis* were collected from the Marine Reserve. In the laboratory I determined total height, maximum width, the distance

between the siphons and wet weight (blotted dry) for these individuals. I then regressed wet weight with the linear measurements to determine the best estimator of ascidian biomass.

Estimates of density and size frequency

Densities and size frequencies were estimated in two ways. At harvested sites, 10 horizontal transects were laid at the tidal height at which I expected to find *P. chilensis*. Each transect was 5 m in length and any *P. chilensis* occurring 10 cm above or below the transect line were counted. Hence each transect corresponded to 1 square metre of rocky substratum. These transects were placed haphazardly in areas spanning a range of exposure to wave action; from exposed vertical rock faces to relatively sheltered pools. Usually, the maximum width of 10 haphazardly selected individuals of *P. chilensis* within each transect was also determined. Measurements were made to the nearest mm. This methodology could not be applied at the Marine Reserve. At this site, quadrats measuring 10x10 cm were placed in pools in the zone in which *P. chilensis* occurs. In these pools, the animals siphons were covered by water and hence they continued to feed. This rendered the bright red lining of the siphons easy to discern and therefore the number of individuals were easily distinguished and counted. Ideally densities should be estimated using quadrats placed at random; hence, my use of pools at this site may bias my estimates of density, although casual observations in areas of the Reserve that lacked pools indicated that this was not the case. Densities in a total of 51 quadrats were recorded in the Marine Reserve, corresponding to a total area of just over half a square meter.

Size frequencies of *P. chilensis* from within the Marine Reserve were determined from monthly samples collected from within 3, 10x10 cm quadrats. These clumps were removed from the rocky substratum and teased apart in the laboratory. The wet weight (blotted dry) of each individual was then determined.

Reproductive activity and estimating recruitment

The size (wet weight) at which individuals become reproductive was determined by dissection in each group of monthly samples from the Reserve. Recruitment was estimated on one occasion (26 February, 1994) within the Reserve and offers a snapshot of recruitment in this species. In the same 10x10 cm quadrats used to estimate the density of *P. chilensis* in the Reserve, I distinguished and counted adults and recruits. I defined recruits as individuals of no more than 10 mm between the siphons, the remaining individuals were recorded as adults.

RESULTS

Determining the best predictor of biomass

The three linear measurements used to predict biomass all offered acceptable fits, with $r^2=0.931$ the lowest value obtained (Table 1). All relationships were best described by power functions, with the exception of maximum width. Here, a logarithmic function offered a slightly better fit ($r^2=0.94$). I decided to use a power function however, as it provided a better estimate of the wet weight of individuals of small maximum width (Fig. 2) and it was these small individuals that dominated the size frequencies of the harvested sites (Fig. 3B). I selected maximum width as the best predictor of biomass as it was the easiest to determine in the field. These data also indicated that defining recruits as those individuals with inter-siphonal measurements of less than 10 mm corresponded to a

wet weight of 5.1 g. Hence, I selected 5 g intervals in subsequent plots of size frequency (Fig. 3).

Estimates of density and size frequency

Densities at the four sites spanned more than 3 orders of magnitude (Table 2). At the two sites that were relatively close to Valdivia, I recorded less than 1 individual per square meter of rocky substratum. This contrasted markedly with the Marine Reserve where densities averaged more than 550 animals per square meter. In addition, only small individuals were found within the harvested areas (Fig. 3). Data pooled from the three harvested sites revealed that individuals rarely exceeded 20 g (1 individual of the 97 examined) and that the mean weight was 2.7 ± 0.4 g (\pm sem, median=1 g). In contrast, *P. chilensis* attained a maximum size of 112 g in the reserve, with a mean weight of 15.3 ± 1.5 g (median=2.25 g). I represented size frequencies of animals from the Reserve as the mean percentage of the population in a given size class in each of 4 months, January to April inclusive (Fig. 3). The total number of individuals examined in each month were 46, 72, 67, and 48 (January to April), respectively.

Reproduction and recruitment

Pyura chilensis achieved reproductive size at between 12 and 13 g (wet weight). All animals above this size in the Marine Reserve were reproductive during January, February and March, with most individuals corresponding to the gonad states III and IV of Cea (1973). Reproductive products were also present in some individuals in

TABLE 1

Functions describing the relationship between wet weight ('y', g) and maximum linear dimensions ('x', mm) for *Pyura chilensis*.
Funciones que describen la relación entre el peso húmedo ('y', g) y las dimensiones lineales máximas ('x', mm) de *Pyura chilensis*.

Measurement ('x')	r^2	Power regression
height (maximum)	0.974	$y=0.001x^{2.612}$
width (maximum)	0.931	$y=0.002x^{2.689}$
inter-siphonal distance	0.956	$y=0.003x^{3.227}$

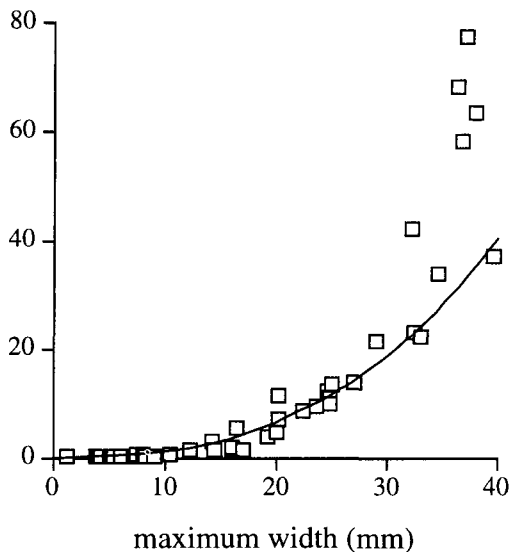


Fig.2. Relationship between the wet weight and maximum width in *Pyura chilensis*. The fitted curve offers an acceptable fit overall (see Table 1), and particularly to the small individuals of interest in this investigation.

Relación entre el peso húmedo y el ancho máximo de *Pyura chilensis*. La curva propuesta ofrece en general un ajuste aceptable (véase Tabla 1) y, particularmente se ajusta bien a los individuos de pequeño tamaño de interés en esta investigación.

April, but they were clearly almost spent (corresponding to gonad state II of Cea, 1973). Animals were probably reproductive prior to January, but this represented the first month in which I collected samples. If I conservatively assume that animals must reach 15 g to become reproductive, then only around 6% of individuals in the harvested populations achieved sexual maturity, this compares with a corresponding average value of $37 \pm 4.8\%$ (\pm sem) for the Reserve.

I observed recruits of *P. chilensis* in 18 of the 51 quadrats examined. Recruits were only observed in the presence of adult conspecifics and were usually attached directly on the test of adult *P. chilensis*. A X^2 analysis led me to reject the null hypothesis that the presence of recruits was independent of the presence of adults ($X^2=12.7$, $P<0.001$, Table 3). The mean density of recruits was 0.9 ± 0.25 quadrat⁻¹ (90 m^{-2}). From the total of 46 recruits that I observed, only five were not in direct contact with adult *P. chilensis* and four of

these were among mussel byssal threads. None of the recruits was more than 2 cm away from an adult *P. chilensis*.

DISCUSSION

Over-exploitation of Pyura chilensis

The data I have presented are consistent with the dramatic over-exploitation of intertidal populations of *P. chilensis*. Densities are between almost 2 and up to 3 orders of magnitude lower at intensively exploited sites than within the Mehuin Marine Reserve. Such dramatic differences in the density of *P. chilensis* over quite small spatial scales have also been observed over a much longer temporal scale within the Marine Reserve (CA Moreno, personal communication). Nevertheless, sizes of individuals are also clearly dramatically depressed outside of the reserve, with probably less than 6% of the individuals achieving a size at which they become reproductive. These data are consistent with the expectation that *P. chilensis* populations close to urban areas are more intensively utilised. It could be argued that the low densities of *P. chilensis* outside the Marine Reserve relates to some biotic or abiotic factor other than human harvesting. I believe that this is unlikely as the occurrence of only small individuals outside of the Marine Reserve is consistent with over-harvesting and large numbers of humans are frequently observed collecting a range of intertidal invertebrates from the harvested areas (Davis, personal observations).

These data probably come as no surprise to Chilean marine scientists, but what is of concern is the likely slow rates of recovery indicated by the recruitment data presented here. Recruitment was only observed in the presence of adults at Mehuin. There may be three reasons for the absence of recruits directly on the rocky substratum. First, larvae may not settle directly onto

³ Davis AR (1994) Neighbours need not be competitors: facilitation of recruitment in subtidal invertebrates (Ascidiacea). 86. Resúmenes, XIV Jornadas de Ciencias del Mar, Puerto Montt.

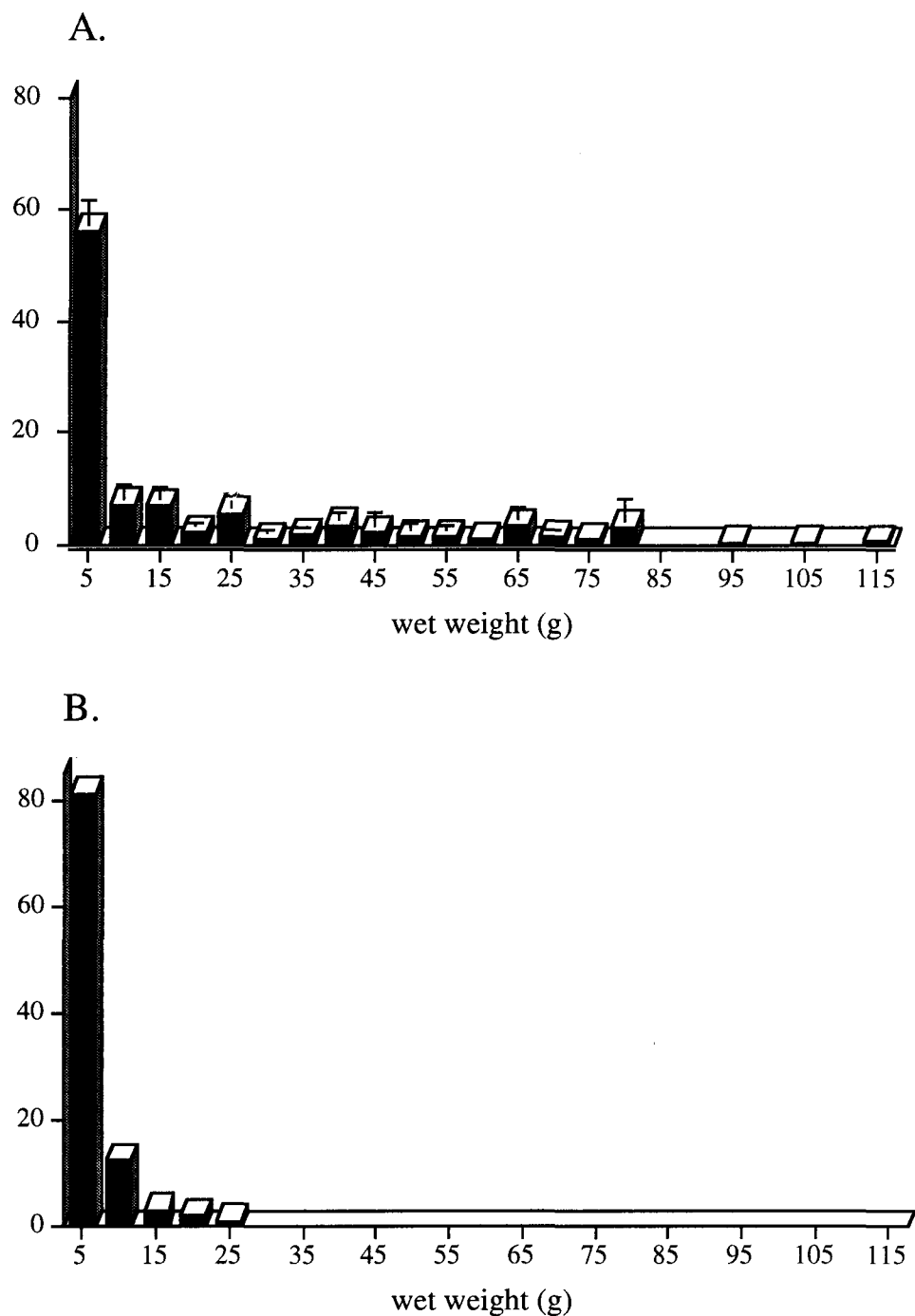


Fig.3: Size frequency of *Pyura chilensis* within the Mehuin Marine Reserve and three harvested locations. A. Size frequencies at Mehuin were determined monthly from January to April, 1994, and are plotted as means ($n=4$ months) with one standard deviation (error bars with a value of less than 1.1 are not displayed). B. In the harvested locations, size frequencies were determined once and data were then pooled for these sites.

Frecuencia de tallas de *Pyura chilensis* en la Reserva Marina de Mehuin y en las tres localidades con intervención humana. A. Las frecuencias de tallas de Mehuin fueron determinadas cada mes, desde enero a abril de 1994, y son graficadas como promedios ($n=4$ meses) y su desviación estándar (las barras de error menores a una no se han graficado). B. En las localidades con intervención humana la frecuencia de tallas se determinó sólo una vez y los datos fueron agrupados.

the rocky substratum, they may, for example, show a preference for the test of adults. Second, recruits may simply arise from asexual buds which are produced on the outer surface of adults and third, settlement may occur in a number of locations, but only those settlers attaching to adults achieve a size at which they would be recorded as recruits. In support of the first hypothesis, laboratory trials with pyurid larvae in Australia indicate that some species will only settle in response to the presence of adult test or other specific cues (Becerro & Davis unpublished data, ³). However, the settlement of *P. chilensis* onto acrylic plates suspended in the water column (Ambler & Cañete 1991) indicates that settlement can ensue directly to the substratum in the absence of adults. In relation to the second hypothesis, some solitary ascidians have been observed to reproduce asexually, but not members of the Pyuridae (Monniot *et al.* 1991). Moreover, electrophoretic data from four species of *Pyura* in Australia indicates that their populations are sexually derived and consist of aggregations of genetically distinct individuals (Ayre & Davis, unpublished data). These findings indicate that the third hypothesis - post-settlement mortality - is probably responsible for the patterns I have reported. I speculate that post-settlement mortality produced by the activities of grazers is the most likely agent of mortality. Ascidian recruits have been found to be dislodged by invertebrates in other studies (Young & Chia 1984, Davis 1988) and the activities of grazers within the Marine Reserve are capable of altering recruitment patterns of sessile invertebrates on artificial plates (JH Zamorano, personal communication). Moreover, grazers were not common on the surface of *P. chilensis* (Davis, personal observations). Paine and Suchanek (1983) identified predatory starfish as important predators of juvenile *Pyura praeputialis*. The starfish, *Stichaster striatus*, was commonly observed in the Mehuin Reserve, but was restricted to areas of extreme wave exposure and was not observed in the moderately exposed areas in which this study was done.

I am not suggesting that the recruitment of *P. chilensis* only occurs in the presence

of adult conspecifics, merely that recruitment will be enhanced under these circumstances. This is reinforced by the data presented in Fig. 3A. In this plot, recruits accounted for almost 60% of the individuals measured, which compares with just 15.6% (90 of 577) from my direct estimates of recruitment (see Table 2). This apparent discrepancy stems from the large number of recruits associated with high densities of adults, as the 10x10 cm plots cleared to generate Fig. 3A were from within large clumps of adult *P. chilensis*.

Observations at the Marine Reserve and nearby Pichicuyin, indicate that *P. chilensis* are capable of living beneath massive aggregations of the tube-dwelling polychaete, *Phragmatopoma virgini* Kimberg, 1867 (Davis, unpublished data). The periodic removal of these polychaete tubes by wave action leaves juvenile and adult *P. chilensis* on the rocky substratum and may provide a focus for subsequent recruitment. Clearly there are a number of testable hypotheses here that would benefit from further research.

Changes in the distribution and abundance of *P. chilensis* may have important implications for other members of the community in which it is an important occupier of space. In general, species which add physical structure to marine systems, such as mytilids, often play host to a large number of associated species (e.g. Witman 1985, Ojeda & Dearborn 1989). It is clear that, at least in the subtidal zone, *P. chilensis* hosts a diversity of organisms (Zamorano & Moreno 1975).

Community-wide effects

Although I provide data for a single exploited species, I believe that there is overwhelming evidence for a general malaise in coastal systems in Chile. The population sizes of many exploited species are clearly depressed. Intertidal systems show dramatic shifts in community structure wrought by humans (e.g. Moreno *et al.* 1984) and I suspect that the same is true of the subtidal zone. How long such high levels of exploitation can be sustained is unclear at this stage. However, there

TABLE 2

Density estimates of *Pyura chilensis* at the four study sites. Estimates were made with 10x10 cm quadrats at Mehuin and 20x500 cm (1 m²) quadrats at the other sites. As data were not normal I have included the estimated median density (sem = standard error of the mean, n = sample size).

Estimaciones de densidad de *Pyura chilensis* en los cuatro sitios de estudio. Las estimaciones fueron hechas con cuadrantes de 10x10 cm en Mehuin y cuadrantes de 20x500 cm (1 m²) en las otras localidades. Debido a que los datos no fueron normales se incluyó la mediana de la densidad estimada (sem = error estándar de la media, n = tamaño de la muestra).

Site	Median	Mean Density (m ⁻²)	n	sem
Playa Rosada	0.3	0.3	0	10
San Ignacio	0.4	0.3	0	10
Pichicuyin	71.5	16.2	82.5	10
Mehuín (Reserve)	576.5	82.7	400	51

may be costs associated with inaction while waiting for information in an area where there are significant unknowns. Myers (1993) states "...there is a premium on a cautious and conservative approach to human interventions in environmental sectors that are (a) unusually short on scientific understanding, and (b) unusually susceptible to significant injury, especially irreversible injury." I would argue that the harvesting of marine resources in many places in the world can be described by these two criteria, but this is particularly the case in Chile. Marine systems in general are characterised by substantial spatial and temporal variations in recruitment success (Connell 1985). For commercially harvested species this is of critical importance (for a Chilean perspective see the recent review by Rodríguez *et al.* 1992). It is clear that exploitation of invertebrates can lead to local extinctions and dramatic reductions in species range (Jamieson 1993). Moreover, many fisheries collapses have occurred and these effects may persist in the long term (Farlinger & Campbell 1992).

The urgent need for marine reserves in Chile

World-wide attention is currently sharply focussed on the assessment of biodiversity and its conservation (for example, see the recent issue of *Ambio* devoted to economic and policy issues relating to biological conservation (Folke *et al.* 1993) and the proceedings of a recent biodiversity

symposium reported in *American Zoologist* (Morse & Thorne 1994)). Chile is clearly playing an active role in the conservation of biodiversity and indeed is utilising innovative techniques to identify shortcomings in its overall conservation strategy for terrestrial systems (Ormazábal 1993).

Efforts to protect terrestrial biodiversity in Chile have been laudable; 80 protected areas with a total area of 13,832,184 ha comprising 18.3% of the national territory (Ormazábal 1993). In contrast, marine systems in Chile are offered scant protection. Chile boasts just 3 marine reserves and only two of these are actively policed. These two reserves comprise less than 1 km of Chile's estimated 10,000 km of coastline; a mere 0.01%. Scientists have been calling for the establishment of marine reserves for almost 20 years (Castilla 1976, 1986, Benoit 1982). The recent suggestion of extending the current boundary of coastal parks into the sublittoral would seem a sensible means of starting (Ormazábal 1993), particularly as the National Parks identified would include a number of biogeographic coastal zones (Brattström & Johansen 1983). The need to initiate this process quickly is emphasized by the recent description of the unusual littoral fauna on the western coast of the Island of Chiloé and the urgent need for its protection ⁴.

⁴ Carvacho A (1994) El litoral de Chiloé occidental y su identidad biogeográfica. 139. Resúmenes, XIV Jornadas de Ciencias del Mar, Puerto Montt.

A stumbling block to the establishment of protected marine areas is the perception that coastal marine systems do not require protection, as organisms are capable of reinvading harvested patches via the dispersal of planktonic larvae (Suchanek 1994). This is undoubtedly the case for many marine species, but relies on a close-enough viable adult population for the production of these larvae. Indeed, the importance of large scale reserves in supplying recruits to a range of fisheries interests has recently been used as a justification for the suggested establishment of an international marine park (McManus 1994). Community integrity and therefore resource viability are unlikely to be wholly determined by recruitment success, however. Superimposed on the arrival of larvae from the plankton are the complex trophic interactions which underpin any marine community. The loss of a small number of species from these communities, depending on which ones are lost, can have dramatic implications for community structure and function (Paine 1966).

Faced with significant unknowns about marine stocks and the continued viability of fisheries resources, I urge Chilean authorities to provide immediate funding for the establishment of protected marine areas. Underwood (1993) concluded that, of the options available for the conservation of exploited flora and fauna, bans on harvesting in designated areas (i.e. marine reserves) was likely to be the easiest to implement and the most effective. Clearly, such protected areas must also be of an

appropriate size. Naturally-bounded areas, such as the whole of a headland, have the advantage of presenting the general public with unambiguous reserve boundaries (Underwood 1993), however, I favour the establishment of reserves on the order of several km in length. This will represent a significant challenge and to be successful will almost certainly need the participation of local people (Wells & Brandon 1993). As current legislation in Chile provides for the establishment of Marine Reserves (CA Moreno, personal communication), they now require only political will and the provision of sufficient resources for their formation. I contend that the benefits flowing from the establishment of these reserves will not only be economic, but educational, scientific and aesthetic. I also believe that funding bodies in Chile should support the establishment of these reserves by providing Chilean marine scientists with resources to establish long-term monitoring programs within and outside these protected areas. This will allow the efficacy of these reserves to be established and monitored. It should also determine whether the criteria developed for effective conservation are indeed being met.

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TABLE III

Estimates of occurrence of recruitment of *Pyura chilensis* in 10x10 cm quadrats
Estimaciones de reclutamiento de *Pyura chilensis* en cuadrantes de 10x10 cm.

		recruits	
		+	-
adults	+	18	17
	-	0	16
		$c^2=12.7$	$P<0.001$

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