



The herbivorous fish, *Aplodactylus punctatus*, as a potential facilitator of dispersal of kelp, *Lessonia trabeculata*, in Chile

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ABSTRACT

Kelp forests are productive marine habitats known to facilitate many trophic and non-trophic interactions. While much of our understanding comes from positive effects of kelp on associated fauna, few studies focus on potential feedbacks that such fauna may provide to benefit kelp. This study aims to analyze the positive interactions between two closely associated fish and kelp species. *Lessonia trabeculata*, the bottom kelp, and the herbivorous fish, *Aplodactylus punctatus*, were sampled at four sites off northern-central Chile to evaluate the following: interactions during the ontogeny of the fish, the availability of reproductive tissue of the kelp blades, the effects of digestion by *A. punctatus* on *L. trabeculata* reproductive tissue, and the viability of zoospores after digestion. Our results show a network of direct and indirect positive interactions between these species. There was a positive correlation in their densities, possibly due to kelp functioning as a refuge and indirect food source. Juvenile *A. punctatus* feed on epifaunal species within the kelp, reducing grazer load and in turn generating a potential positive indirect effect on the kelp. Adult herbivorous fish consume kelp tissue as sustenance; when this kelp tissue is reproductive, digestion reduces epiphytic algal densities on the sori. Zoospores were found to remain viable after digestion, and microscopic sporophytes were produced at rates similar to those of undigested reproductive tissue. We conclude that positive links between *A. punctatus* and *L. trabeculata* occur along the geographic range distribution of both species and that this herbivorous fish could serve as a dispersal mechanism for *L. trabeculata*. To our knowledge, this is the first study that suggests that an herbivorous fish may have the potential to be a facilitator of kelp zoospore transport, and this may contribute positively to the recovery of natural kelp populations that are being intensively harvested in the region.

1. Introduction

Kelps, brown algae belonging to the order Laminariales, support abundant and diverse associate macrofaunal assemblages (Dayton, 1985; Steneck et al., 2008; Villegas et al., 2008). The benefits provided by kelp populations to associated species allow entire ecosystems to exist and therefore, they are considered foundation species (Bodkin, 1988; Stachowicz, 2001). These seaweeds provide shelter from predators (Carr, 1991), improve habitat quality by ameliorating environmental stress (Stachowicz, 2001; Teagle et al., 2017), and expand niches in which species can live (Dayton, 1985; Bruno et al., 2003; Graham, 2004; Steneck et al., 2008). Several fish species use kelp forests as nursery habitats, where survival is enhanced (Carr, 1991;

Anderson, 1994; Carr, 1994). Moreover, kelp provide food both directly – from their tissue – and indirectly – from associated understory algae for herbivorous animals (Andrew and Jones, 1990) as well as the organisms that live on and within the kelp (Norderhaug et al., 2005; Koenigs et al., 2015; Miller et al., 2015), forming exceptionally complex food webs in these systems (Graham, 2004).

In temperate ecosystems, there are few examples of how fauna can positively influence their habitat (Hay et al., 2004). Witman (1987) established that mussel beds benefited from sea urchin grazing on kelp growing on the mussel shells, reducing the impact of storms due to dislodgment. Furthermore, Bertness and Leonard (1997) found that mussel beds allow the stabilization of the substrata while providing nutrients from their waste to saltmarshes. These examples indicate that

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positive interactions between associated fauna occur in both sessile and mobile species. Despite the few obligate fish species in kelp forests, positive effects of fish on kelp species have been demonstrated via indirect density-mediated effects by reducing grazer abundance (i.e., urchins and mesograzers) (Cowen, 1983; Davenport and Anderson, 2007; Newcombe and Taylor, 2010), and via trait-mediated effects in which the presence of predators influences the impact of grazers on host seaweed (Byrnes et al., 2006; Pérez-Matus and Shima, 2010). These interactions generate important links between kelps and associated species. Positive species interactions and their influence on community structure have been previously observed (Bruno et al., 2003; Hay et al., 2004) and habitats dominated by kelps are ideal to study these interactions because they are known to ameliorate environmental stress and establish connections among species (Bertness and Leonard, 1997; Byrnes et al., 2011).

Herbivory is an important interaction between flora and associated fauna, and the effects are influenced by different factors (Poore et al., 2012), including the type of herbivore (Jones and Andrew, 1990; Aguilera, 2011), morphological and/or chemical resistance to grazing (Jormalainen et al., 2005; Cerda et al., 2009), and environmental gradients (Vanderklift et al., 2009; Taylor and Schiel, 2010). In terrestrial systems, selective pressures from herbivory have resulted in the development of chemical or physical defenses to deter grazing, or conversely, adaptations that encourage grazing to enhance dispersal, such as seeds that are resistant to digestion (in some species, germination rates are elevated following digestion: Traveset and Verdú, 2002; Tiffney, 2004). Mammals and birds are the most common species that act as seed dispersers, but recent studies have added terrestrial invertebrates to the list (Duthie et al., 2015). In marine environments, the study of dispersal processes by biological vectors is complicated due to the great diversity of algal reproductive strategy (i.e., differentiated reproductive/vegetative tissue, asexual reproduction (Santelices, 1990)). Algal resistance to digestion depends on algal life history and traits (e.g., feeding mode) of the herbivorous species. Reproductive and vegetative tissues are able to regenerate or germinate after digestion by invertebrates among opportunistic algae that use asexual reproduction and have low complexity in their tissues (Santelices et al., 1983; Santelices and Ugarte, 1987; Cabral de Oliveira, 1991). Macroalgae with greater complexity (i.e., differentiated vegetative and reproductive tissues) have lower post-digestion survival rates due to the inability to regenerate from vegetative tissue and/or by the differentiation of reproductive tissue, which is less likely to be consumed (Santelices et al., 1983; Santelices and Ugarte, 1987; Skern et al., 2003).

As in terrestrial ecosystems, the dispersal abilities of algae by a biological vector have attracted the attention as it may represent benefits to macroalgae distribution dynamics. Buschmann and Bravo (1990) described preferential feeding of the amphipod, *Hyale media*, on reproductive structures of algae such as *Mazzaella*, facilitating spore release and dispersal when spores adhere to their legs (Buschmann and Santelices, 1987; Buschmann and Bravo, 1990). In tropical marine environments, Phaeophyceae (including foliose, filamentous, and crustose types) and Rhodophyta (corallines and filamentous turfs) have the capacity to reattach and grow after digestion by parrotfishes and surgeonfishes, potentially increasing dispersion (Vermeij et al., 2013; Tâmega et al., 2015). In temperate environments, Paya and Santelices (1989) found that an opportunistic alga resisted digestion by an intertidal clingfish. This pattern is also present on marine gymnosperms such as seagrasses, which also resist fish herbivory (Sumoski and Orth, 2012). Since some algae cannot survive digestion by invertebrate species, herbivorous fishes can be biological vector for algal dispersal due to the differences in the digestion processes, assimilation capabilities and greater mobility (Choat et al., 2002; Clements et al., 2017).

Kelps (order Laminariales) are globally distributed brown seaweeds, characterized by a complex life cycle with motile flagellated zoospores that are capable of short distance dispersal (but see *Macrocystis pyrifera*: Macaya et al., 2005). The short duration of zoospore viability (typically

hours) is generally considered to limit average dispersal distances to the scale of several meters to up to a kilometer (Reed et al., 1992; Gaylord et al., 2004; Steneck et al., 2008). Past studies have indicated that dispersal success is determined by a combination of physical and biological factors (Steneck et al., 2008). All Laminariales species have a diplohaplontic heteromorphic life cycle, where zoospores are released into the water column and must find suitable substrate in order to attach, germinate, form into male and female gametophytes, and undergo fertilization, for successful recruitment to occur. High zoospore settlement densities (> 1 zoospore per mm^2 ; Reed et al., 1992) are critical for kelp recruitment success, and densities have been shown to decline with increased distance from adult individuals (Buschmann and Santelices, 1987; Gaylord et al., 2004). Therefore, processes that increase the aggregation and dispersion of zoospores (e.g., aggregating, rafting, herbivore-assisted dispersal; Santelices and Paya, 1989; Santelices and Ugarte, 1987; Thiel and Gutow, 2005) may enhance the chances for kelp to colonize new rocky areas.

The goals of the present study were to explore the positive interactions between the most abundant herbivorous temperate reef fish and the subtidal kelp *Lessonia trabeculata*. We also conducted a series of mensurative and manipulative experiments to assess the influence of *Aplodactylus punctatus* in kelp beds by: (1) verifying the overlapping distributions and associations; (2) analyzing ontogenetic changes in the diet of *A. punctatus*; and (3) testing the effects of digestion by *A. punctatus* on *L. trabeculata* reproductive tissue and viability of zoospores, potentially demonstrating *A. punctatus* as a disperser of *L. trabeculata*.

2. Material and methods

2.1. Kelp (*L. trabeculata*) and fish (*A. punctatus*) distribution and density

The subtidal rocky habitat on the cold-temperate southeast Pacific coast is dominated by *Lessonia trabeculata* (Villouta and Santelices, 1986), which is distributed from Peru (14°S) to Ancud, Chiloe Island, Chile (42°S) from 0.5 to 30 m depth with massive holdfast and multiple stipes reaching up to 2.5 m (Villouta and Santelices, 1986). *Lessonia trabeculata* is a perennial species that produces its reproductive tissue on ordinary blades and is present year-round with seasonal reproductive peaks, possibly associated with low light intensity, temperature, and high nutrient availability (Tala et al., 2004; Murua et al., 2013).

The herbivore “jerguilla” (“marblefish” in English) *Aplodactylus punctatus* (Valenciennes, 1832) is distributed from Paita (5°S Peru) to Gulf of Arauco in Chile (36°S) (see Froese and Pauly, 2016). However, in this current study we found populations south of this southernmost distribution boundary. We sampled and recorded the presence of this species in kelp (*L. trabeculata*) beds (8–15 m depth). This fish is the most abundant herbivore in kelp beds (Cáceres et al., 1993; Pérez-Matus et al., 2007, 2017). Their diet is influenced by food availability (Cáceres et al., 1993; Pérez-Matus et al., 2012) and adult diets are composed of 75% kelp (*L. trabeculata*) and 10% other algae (i.e., *Gelidium*, *Ulva*, and corallines) (Benavides et al., 1994; Pérez-Matus et al., 2012). Juveniles (< 30 cm in total length) of this species consume mainly epifauna such as amphipods, bryozoans, hydrozoans, polychaetes, and porcelain crabs as well as epiphytes and other turf algae (Cáceres et al., 1993; Benavides et al., 1994).

During austral fall (March 27 through April 11) and spring (October 30 through November 13) 2012, we monitored 4 kelp-dominated semi-exposed to wave action sites of the north and central Chilean coast spanning ~350 km to quantify *L. trabeculata* and *A. punctatus* densities. The sites included El Francés (30.09°S, 71.37°W), Punta Talca (30.87°S, 71.69°W), Quintay (33.19°S, 71.70°W), and Algarrobo (33.36°S, 71.69°W). Visual surveys using SCUBA were conducted at each site, employing four 50 m transects running perpendicular to the coast at each site between 5 and 20 m depth. Fish abundances and the total

length (TL) of each fish was visually recorded for 2 m on either side of the transect. In addition, the position and abundance of each fish relative to kelp blades and stipes, holdfasts, and/or outside the kelp stands was visually recorded (Pérez-Matus et al., 2007).

Differences in densities of reef fish (*A. punctatus*) and kelp (*L. trabeculata*) among seasons and sites were analyzed using general linear models (GLMs). We conducted three analyses separately using abundance of juvenile fish, adult fish (*A. punctatus* only), and kelp (*L. trabeculata* only). The response variable was based upon counts that had unequal variances and non-normally distributed errors. Data were over-dispersed for all analyses (density of juveniles, residual deviance model = 45.38 on $df = 24$; total density of fish residual deviance model = 67.44 on $df = 24$; and kelp density, residual deviance model = 157.08 on $df = 24$) and were corrected using a quasi-Poisson distribution (Crawley, 2007). To determine if fish density (juveniles and adults) was correlated with kelp density, a Spearman-rank correlation test was conducted using data pooled by seasons (mean of transect in each season).

Finally, in order to evaluate the differences in abundance of juveniles and adult *A. punctatus* in kelp blades/stipes, holdfasts, or outside of the kelp stands, we used a permutational multivariate analysis of variance (PERMANOVA: Anderson, 2001). We used different size ranges (every 10 cm in TL) and study site as fixed factors on the abundance of individual *A. punctatus* and each of the three habitats categories (kelp blades and stipes, holdfasts, and/or outside stands) as response variables. All data analyses were performed in R Core Team, 2015 (Version 0.99).

2.2. Effect of fish herbivory on kelp reproductive tissue

In order to evaluate the presence/absence of kelp zoospore in gut contents of adult *A. punctatus*, we collected two individuals of *A. punctatus* (mean size of 39.5 ± 1.5 cm TL) using a spear-gun in a kelp bed at 8 m depth near Valparaíso ($32^{\circ}52'S$, $71^{\circ}31'W$) at the end of austral summer, March 24, 2011. In the laboratory, we removed the gut of each specimen and divided long digestive tract into two portions (anterior, posterior) and extracted all the contents of the excised portions. Additionally, pieces of the kelp (*L. trabeculata*) were separated using a dissecting microscope. At the same site where the *A. punctatus* were caught, we removed blades of *L. trabeculata* and cut off pieces of visible reproductive tissue to serve as our control. All algal tissues from the anterior and posterior portions of the gut, and the control tissues were evenly distributed to 10 different ($n = 30$), 12 mL petri dishes with filtered seawater. The tissue pieces remained in the petri dishes for 5 d under 12:12 light:dark period. After 5 d, the tissue pieces were removed and the presence/absence of zoospores were monitored using a microscope. This served as preliminary study that enabled us to determine if zoospores were present inside the digestive tract of the collected herbivorous fish.

According to our preliminary study, kelp zoospores were present in *A. punctatus* and our control in petri dishes (see appendix S1, Fig. S1). In order to quantify the amount of structural damage on kelp tissue and rate of kelp zoospores germination after passing the digestive tract of this herbivore, feeding assays were conducted on April 28, 2015 and lasted 4 consecutive d (96 h). We evaluated the effects of *A. punctatus* on reproductive tissue of *L. trabeculata* in laboratory mesocosms using 3 circular, 800 l tanks (1.5 m diameter and 0.5 m high). Each tank was covered with a black mesh (~0.5 mm mesh size) to avoid overheating of the mesocosm. 9 adult (mean 39.6 cm \pm 2 SE in total length [TL]) *A. punctatus* were collected using a knotless experimental gill net (20 \times 5 m; 30 mm mesh size) placed in the kelp bed (~15 m depth) for 25 min at Algarrobo ($33.36^{\circ}S$, $71.69^{\circ}W$). Individual *A. punctatus* were collected live and transported to the laboratory in sealed containers with aerated seawater. Once in the laboratory, individuals were acclimatized in 800 L flow-through circular tanks (1.5 m diameter and 0.5 m high) for 24 h without food then transported to experimental

mesocosms.

Blades with reproductive tissue from different adult individuals of *L. trabeculata* with 20–30 cm holdfast diameters were collected; the stipe internodes were cut using a diving knife from nearby sites (Punta de Tralca, $33^{\circ} 25' S$, $71^{\circ} 42' W$). Blades were placed in a mesh bag and transported to the laboratory in a 40 l cooler with seawater at ambient temperature. In the laboratory, blades were attached using epoxy resin to small boulders (30 cm diameter). 3 boulders with 10 blades were placed in 3 independent mesocosm tanks with running seawater ($n = 3$; see appendix S1, Fig. S2). 3 *A. punctatus* individuals were added to each mesocosm as preliminary observations concluded that this fish species did not feed when placed alone in mesocosm, while in groups of three individuals fed regularly in captivity and displayed reduced signs of stress. Fish individuals started feeding immediately after kelp blades were provided. A singular mesocosm served as the control treatment and contained kelp blades without any fish individuals. This autogenic control is referred hereafter as undigested reproductive tissue. Distinctive bite marks by *A. punctatus* were observed on the kelp blades and these corresponded to similar marks observed in the field (see appendix, Fig. S2). Thus, we were certain that kelp tissue was directly consumed by *A. punctatus* in captivity. Digested reproductive tissue was collected each day by siphoning the bottom of the tanks and sieving (500 μ m) the fecal matter produced by the fish (onwards digested reproductive tissue). At the end of the experiment, > 90% of the kelp tissue provided was consumed by *A. punctatus*.

To evaluate the structural and mechanical damage generated by grazing and digestion of *L. trabeculata* reproductive tissue, 10 digested and 10 undigested reproductive tissue samples were sectioned for microscopic analysis by performing 5 transverse cuts. Thin sections of reproductive tissue were fixed on a slide and visualized using an Olympus CX21 microscope and microimaging camera (CMOS 5Mp CO-5). Additionally, the presence and absence of epiphytes on the tissue surface was also estimated for each transverse section. The presence/absence of epiphytic algae was compared using a GLM with a quasi-binomial error distribution because the data was over-dispersed (residual deviance model = 102.27, $df = 97$) and a logit link function.

To evaluate the viability of *L. trabeculata* zoospores following passage through the digestive tract, feeding assays were repeated in May 2016 (same design as above). Digested and undigested sorus tissues were obtained from the mesocosms siphoned and rinsed with seawater. One gram of each tissue was placed in Petri dishes with 12 ml of filtered seawater ($n = 6$ replicates of each). The tissue remained in the dishes for 24 h before being placed in a solution of filtered Provasoli's enriched seawater (PES), and the medium was replaced weekly. Reproductive tissues from both treatments were then cultured to compare the timing of sporophyte development between digested and undigested reproductive tissue. Digested material was composed of both vegetative and sorus tissue, and could not be separated after passing through the fish's digestive tract. Thus, in order to compare sporophyte densities per gram between the digested and undigested tissues, sporophyte densities from undigested tissue were standardized to the percentage of reproductive tissue presenting blades of *L. trabeculata* (20% approximately, Ruz, 2014 appendix S1, Fig. S4). Cultures were maintained for 5 weeks in a basin with running seawater (11.5 $^{\circ}C$) with a 12:12 light:dark cycle and 40 μ mol photons. m^{-2} . s^{-1} and monitored weekly for sporophyte production using an inverted microscope (Motic® AE2000). All the sporophytes per Petri dish were counted only and standardized per gram of kelp tissue. We used a GLM with a quasi-Poisson error distribution for over-dispersed data (residual deviance model = 155 on $df = 10$) to compare the sporophyte counts per gram of undigested and digested kelp tissue. The model fit was checked with an F-test analysis of variance (ANOVA) for analysis of deviance (Dev) vs. the degrees of freedom (Hastie and Pregibon, 1991).

3. Results

3.1. Kelp (*L. trabeculata*) and fish (*A. punctatus*) and density and distribution

This study found similar latitudinal and bathymetric distributions of *Aplodactylus punctatus* and *Lessonia trabeculata*. We recorded *A. punctatus* in kelp beds near Bahía Mansa, Puerto Montt (40° 38' S) and Ancud, Chiloe Island (41° 45' S–73° 54' W), south of the reported geographic distribution range of these species; we therefore extended the reported southern distribution range of this fish species (see appendix 2, Video S1). Kelp densities were positively correlated with *A. punctatus* juvenile and adult densities across the four study sites sampled. Positive effects of kelp were statistically significant for juvenile fish density (juvenile Spearman, $\rho = 0.7$, $p = 0.03$; Fig. 1a), but not adults (adult fish, Spearman, $\rho = 0.2$, $p = 0.7$; Fig. 1a). *Aplodactylus punctatus* were observed at all stages in all sites and the size of the individuals observed ranged from 3 cm (young of the year) to 55 cm (adults). Therefore, juvenile (omnivorous) and adult (herbivorous) life stages occurred at all sites. In terms of habitat used within kelp blades, stipes or holdfast, or outside kelp stands, we found no effect of site (PERMANOVA, $df = 3$, $pseudo-F = 1.83$; $p = 0.08$), but there was an effect of fish ontogenetic stage (size classes) in relation to patterns of habitat use (PERMANOVA, $df = 1$, $pseudo-F = 3.54$; $p < 0.01$). We found no interaction between size range and sites in patterns of habitat use (PERMANOVA, $df = 3$, $pseudo-F = 0.69$; $p = 0.71$). Juvenile fish individuals between 3 and 20 cm in TL were primarily associated with the blades and stipes, whereas individuals larger than ~20 cm in TL were generally observed around the holdfast and outside of the kelp

stands (Fig. 1b).

Aplodactylus punctatus was observed at all sites but densities were highest at Mineral de Talca and Quintay, which exhibited a 2-fold increase in abundance relative to El Francés and Algarrobo (see appendix S1, Fig. S3a). Significant differences among sites were observed only in juvenile fish densities (GLM, $df = 3$, $Dev = 30.35$, $p = 0.01$), without season (GLM, $df = 1$, $Dev = 0.01$, $p = 0.92$), and interaction effects (GLM, $df = 3$, $Dev = 48.5$, $p = 0.21$) (see appendix S1, Fig. S3a).

Kelp abundances were also higher at Mineral de Talca and Quintay, with a 5-fold increase in abundance (10 and 12 individuals per 100 m^{-2} , respectively) relative to El Francés and Algarrobo. Differences in density were statistically significant among sites (GLM, $df = 3$, $Dev = 408.06$, $p < 0.0001$) and seasons (GLM, $df = 1$, $Dev = 69.37$; $p < 0.001$), with the interaction between these two factors also being significant (GLM, $df = 3$, $Dev = 55.23$; $p = 0.01$). The differences between seasons were driven primarily by an elevated density of young kelp recruits (< 10 cm holdfast) in the fall at Mineral de Talca (see appendix S1, Fig. S3b).

3.2. Effects of digestion on *L. trabeculata* reproductive tissue

Our preliminary field essay indicated that the proportional presence of kelp zoospores differed between the posterior and anterior portion of *A. punctatus* digestive tract and our control tissues. Kelp zoospores were present in 70% of the anterior portions of the digestive tract of *A. punctatus*, 100% of the posterior portions, and 50% of the control in Petri dishes (see appendix S1, Fig. S1). On the other hand, comparisons of *L. trabeculata* reproductive tissue digested by fish individuals and undigested tissue indicated that the digestion process did not generate

a) Kelp-fish Correlation

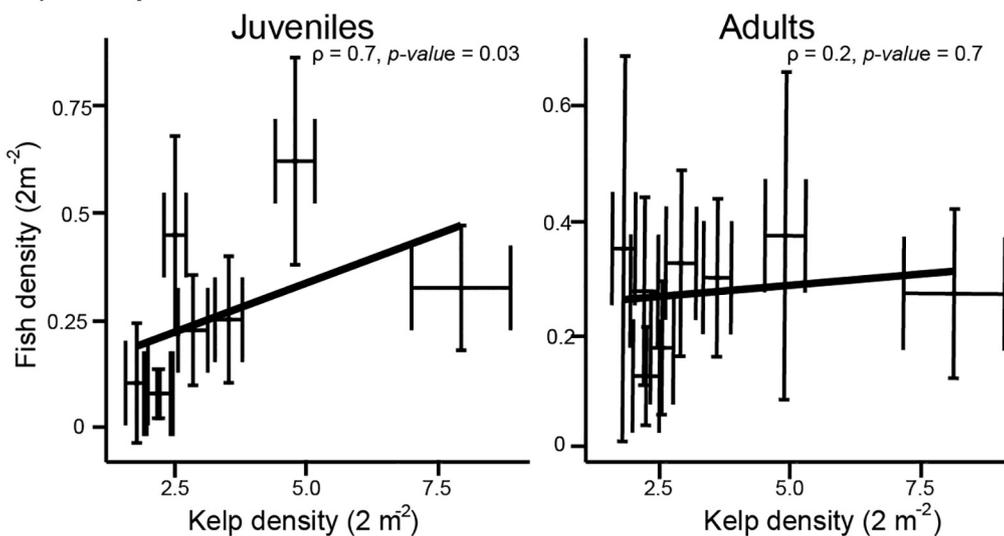
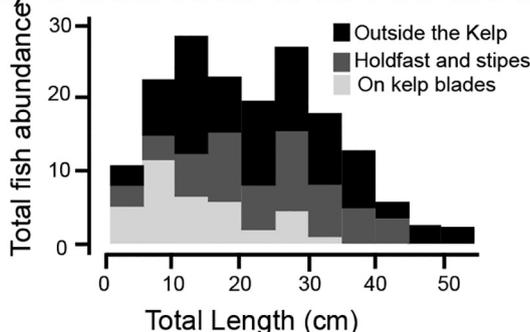
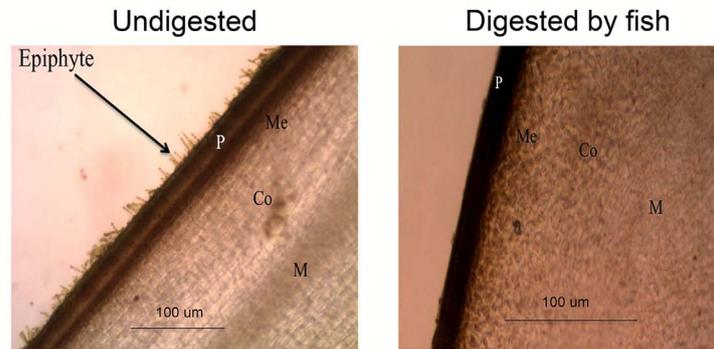
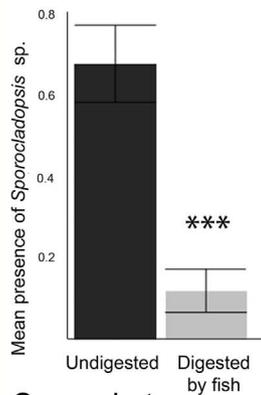


Fig. 1. (a) Correlation of mean (\pm SE) densities of juvenile and adult *Aplodactylus punctatus* and *Lessonia trabeculata* among sites (b) size (cm) frequency distribution of total fish abundance *Aplodactylus punctatus* in all sites and seasons relative to the kelp (blades, holdfast) sporophylls and outside a stand of kelp.

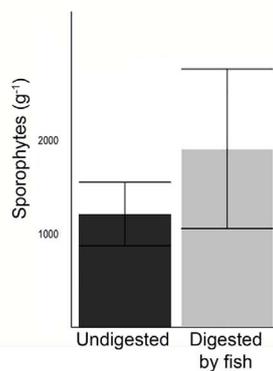
b) Distribution of fish on the kelp



a) Epiphyte load



b) Sporophyte



2.5 weeks



5 weeks

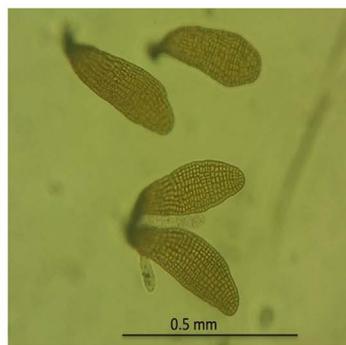


Fig. 2. Effect of fish digestion (or lack thereof) on (a) mean presence of the epiphyte, *Sporocladopsis* sp. on the reproductive tissue of *Lessonia trabeculata*. *** indicate statistical significant differences ($p < 0.001$). Photomicrographs illustrating the cell layers through transversal cuts of digested and undigested sporophyll tissue: medulla (M), cortex (Co), meristoderm (Me), paraphyses (P). The arrow depicts the epiphyte. (b) Sporophytes abundance per gram of tissue from digested and undigested kelp in laboratory culture. Paired images highlight similarities in the timing and viability of sporophyte development from digested and undigested sporophyll tissue at 2.5 and 5 weeks duration.

apparent structural damage to kelp cell layers. Tissues layers such as the medulla, cortex, meristoderm, and paraphyses were structurally intact (100% of the time) in the digested tissue and indistinguishable from those same tissue layers in undigested (i.e., control) tissue (Fig. 2a). In contrast, epiphytic algae were ~6 times more abundant on undigested than on digested tissue (GLM, $df = 2$; $Dev = 32.4$, $p < 0.001$; Fig. 2a).

3.3. Viability of digested zoospores

Zoospores released from kelp tissue that passed through the digestive tract of *A. punctatus* were viable (see appendix S1, Fig. S1) and did not differ from the undigested control tissue neither in terms of the timing and maturation of zoospores and gametophytes nor in the production of microscopic sporophytes (monitored to 5 weeks; Fig. 2b). Sporophyte densities produced in culture were not significantly

different between the undigested ($1209.5 \text{ g}^{-1} \pm 341.2$) and the digested material ($1907.5 \text{ g}^{-1} \pm 854.0$; GLM, $df = 1$; $Dev = 15,297$, $p = 0.43$; Fig. 2b) after controlling for the fraction of total kelp tissue consumed that was composed of reproductive tissue (standardized density in undigested tissue = sporophyte density/0.2; sorus tissue comprised 20% of the blade area). Thus, kelp zoospores were fully viable following digestion, resulting in sporophyte densities similar to those from undigested control tissue.

4. Discussion

Lessonia trabeculata forests found on the Pacific coast of southern Peru and Chile are an important near-shore habitat. Our results highlight multiple direct and indirect positive interactions between the kelp and the fish associated with them (summarized in Fig. 3). The reef fish *Aplodactylus punctatus* was found to inhabit these forests throughout

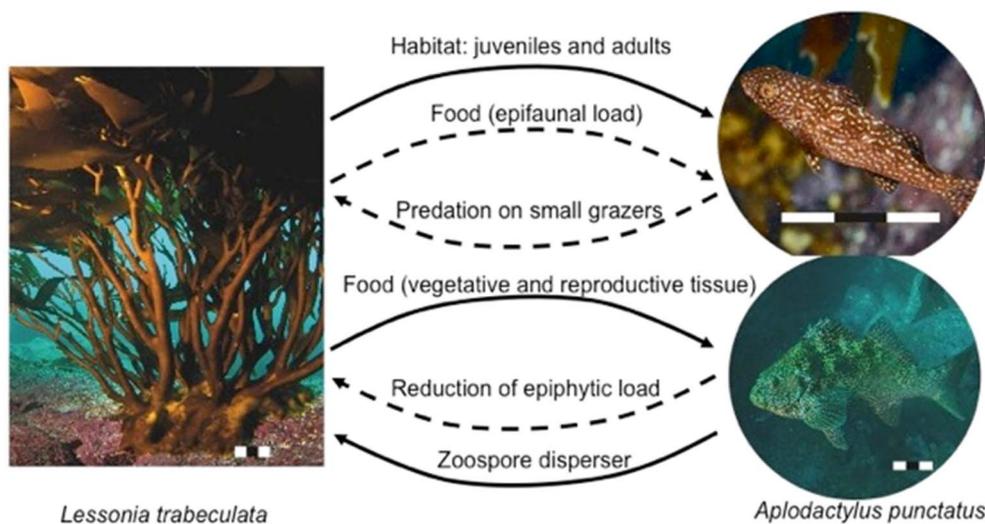


Fig. 3. Schematic representation of the potential positive interactions between the kelp *Lessonia trabeculata* and the herbivorous fish *Aplodactylus punctatus*. Solid and dashed lines indicate positive direct and indirect effect, respectively. Habitat is provided by *L. trabeculata* to juveniles and adult *A. punctatus*. Blades serve as site for recruitment and dense beds of *L. trabeculata* increase density of this temperate reef fish (solid line). Kelp provides food indirectly via epifaunal load for juvenile fish (dashed line), which predate on small grazers (dashed line). Kelp is food to adult *A. punctatus* that grazed on vegetative and reproductive tissue (solid line). Through digestion, *A. punctatus* reduce the species-specific epiphytic load (dashed line) *A. punctatus* can serve as potential zoospore disperser. See results sections for more details on each path. Scale bar: ~5 cm. Photos: A. Pérez-Matus.

their ontogeny, using *Lessonia trabeculata* at different stages for different purposes. Upon close examination of this relationship, it was observed that this herbivorous fish relies on *L. trabeculata* for food and habitat along their geographic distribution range, but may also contribute to the population persistence of *L. trabeculata* (Fig. 3).

The *Lessonia trabeculata* kelp forest supplies direct benefits to juvenile *A. punctatus* by providing habitat and refuge space, an idea supported by the spatial distribution of juveniles among kelp microhabitats where juveniles exhibited a strong association with the complex structural components of the kelp (i.e., blades and stipes). Habitat features have been shown to widely influence the structure of marine reef-fish assemblages, including macroalgal habitat (reviewed by Ebeling and Hixon, 1991; Pérez-Matus et al., 2017). Positive associations between algal structure and fish abundances have been demonstrated experimentally in a number of systems (Davenport and Anderson, 2007; Pérez-Matus and Shima, 2010). In addition to providing shelter, juveniles feed on the associated epifauna of *L. trabeculata*, including hydrozoans, polychaetes, bryozoans, bivalves, and amphipods (Benavides et al., 1994). Examples of positive indirect effects of fishes on seaweeds typically involve a reduction of grazing impacts via consumption of kelp mesograzers (Duffy and Hay, 2000; Davenport and Anderson, 2007), but some mesograzers consume epiphytes leaving no damage on their host algae (see Duffy, 1990). Consumption of mesograzers, such as amphipods and isopods, can result in enhanced kelp growth (Davenport and Anderson, 2007). Our field-based studies confirm that juveniles preferentially inhabit kelp blades where they feed (based on dietary information of this species), thus the kelp provides refuge and food to juvenile *A. punctatus* (Fig. 3).

As previously mentioned kelp forests play a key role throughout the ontogeny of *A. punctatus*. Juvenile fish were frequently observed on the blades, where individuals may have a heightened ability to avoid predators and increased availability of food sources. Larger individuals remained in the forest, but moved out of the kelp stand and consumed the blades (vegetative and reproductive tissue) directly. Associations between both kelp and the fish distribution and densities were confirmed and may serve as an excellent model to test hypotheses of positive interactions.

Adult *Aplodactylus punctatus* feed directly on the kelp blades (Benavides et al., 1994; Cáceres et al., 1994; Pérez-Matus et al., 2012), which has subtle effects on the kelp since fish only consume a portion of blade tissue. Consumption of blades from *L. trabeculata* includes reproductive tissue, and our results revealed that post-digestion reproductive tissue showed a reduction of the epiphyte *Sporocladopsis* sp. in the paraphyses and sporangia surfaces. This filamentous epiphyte is specific to reproductive tissue in *Lessonia* spp. due to the rough texture

of the reproductive tissue compared to the smooth texture of the vegetative tissue (Martinez and Correa, 1993). The presence of *Sporocladopsis* could inhibit zoospore release (Martinez and Correa, 1993) and the removal of these epiphytes upon passage through the digestive tract of *A. punctatus* may aid zoospore availability.

Difficulties in the assimilation of food (i.e., kelp tissue) could lead to an increase in *A. punctatus* consumption rates and to the probability of consuming reproductive tissue of multiple kelp individuals, thereby enhancing zoospore release, dispersal, and fertilization success, which require high zoospore densities (Reed et al., 1992; Muth, 2012). Based on this, we conclude that hindgut fermenters such as *A. punctatus* are efficient in assimilating the digested epiphyte load on reproductive tissue, while the sporangium is left intact after passing through the digestive tract of *A. punctatus*. Interestingly, herbivores who browse on epiphyte and host algae are described as a feeding mode that corresponds to the ‘peanut butter and nutritionally unsuitable crackers’ analogy (Cummins, 1974), a device used to explain the mechanism used by aquatic insects to harvest microbial biomass from leaf litter in streams. Furthermore, in a recent review, Clements et al., (2017) expanded this analogy to several other browsers such as parrotfishes in which the crackers correspond to seagrasses and macroalgae (Phaeophyceae), and the peanut butter corresponds to epiphytes (including the protein-rich cyanobacteria). This suggests that fish browsers seek the protein-rich components of epiphytes that have value also in temperate ecosystems (Johnson et al., 2017). In this sense, the evidence provided by gut contents studies on herbivores (Benavides et al., 1994; Pérez-Matus et al., 2012) may have underestimated the importance of epiphytes as a food source while also overestimating the importance of host macrophytes that remain in herbivore guts.

In temperate reef ecosystems, herbivorous fish play an important role in determining the biological structure of reef communities (Horn, 1989). Nonetheless, the assimilation of algal material in the diet of marine herbivores is poorly understood in comparison to terrestrial herbivores (Clements et al., 2009). Studies conducted with another aplodactylid fish (Mountfort et al., 2002) indicated that values of hindgut fermentation are among the lowest recorded for any temperate reef fish herbivore. Here, we show that post-digestion reproductive material of *L. trabeculata* remained viable, as it was revealed from both field and mesocosm experimental studies conducted herein. Moreover, spores are able to produce microscopic sporophytes within similar timelines and at similar densities to undigested material. Gut passage times in the digestive tract (Sumoski and Orth, 2012) of *A. punctatus* can be up to 20 h (Benavides et al., 1994), which may influence the dispersion of kelp zoospores depending on the scale of daily movements of the fish. Unfortunately, the home range and movement behaviors of *A.*

punctatus are not known; however, in a 20-h period we assume the fish could move and increase zoospore dispersal over passive processes. Fish may be more efficient dispersers than other kelp herbivores, such as amphipods and urchins, because of the mobility differences between fish and urchins. Additionally, urchins may not be as useful as vectors of zoospore dispersers, since seaweed propagules have little chance of reaching adulthood in urchin barrens (Perreault et al., 2014). Further research using telemetry tracking is needed to understand the fish movements within kelp forests.

Our results suggest that post-digestion viability of *L. trabeculata* zoospores provides the potential for *A. punctatus* to act as a zoospore disperser. Large variability in sporophyte densities was expected in the digested material because it was composed of vegetative and reproductive tissue. Zoospores were artificially aggregated and sporophyte densities measured in the laboratory should not be assumed to reflect natural densities in the field. However, the lack of differences in the timing or magnitude of sporophyte development among treatments highlights the ability of zoospores to remain viable following digestion. Additionally, *A. punctatus* may facilitate settlement and recruitment by: (1) increasing the sinking speed of zoospores contained in feces compared to those released directly into the water column; (2) reducing mortality of free-floating zoospores that are at risk of predation; (3) mixing zoospores from multiple kelp individuals during fish foraging bouts, thereby increasing fertilization success and genetic diversity; (4) and enhancing zoospore aggregation on the benthos at densities above the threshold required for sexual fertilization of the gametophyte stage (Reed et al., 1991, 1992). We further suggest that additional nutrients can be supplied via fish feces directly (see Bertness and Leonard, 1997).

Other examples of the resistance of marine macrophytes to digestion include the consumption of fragments of opportunistic green and red algal species by fish (Gaylord et al., 2004; Paya and Santelices, 1989) and the seeds of seagrass (Sumoski and Orth, 2012). In both examples, the propagules pass through the digestive tract and do not require any additional steps (gametogenesis and fertilization) for successful viability, recruitment, or regeneration. In contrast, the complex life history of kelps – characterized by obligate alternating generations between gametophyte and sporophyte stages – requires that sensitive microscopic stages are resistant to digestion. The benefits of herbivore-assisted zoospore dispersal in kelp forests are challenging to measure empirically, because it is difficult to identify in the field which kelp recruits are generated via passage through the gut of *A. punctatus* or passive release from adult kelp. However, this study provides the first step by unequivocally demonstrating the zoospore viability of digested reproductive material relative to undigested material in a laboratory setting.

Harvesting of *L. trabeculata* and reef fishes (including *A. punctatus*) is intense in the south Pacific coast (i.e., Chile contributed to > 40% of world export of kelp; Vásquez, 2008; Krumhansl et al., 2016; Pérez-Matus et al., 2017). Any facilitation of recruitment via fish could serve population sustainability and persistence, especially because kelp harvesting significantly increases the fragmentation and isolation of Chilean kelp forests (Vásquez, 2008; Krumhansl et al., 2016). Similarly, in this study, the balance of positive direct and indirect effects highlights a complex relationship between a temperate herbivorous fish and their host, *L. trabeculata*, which warrants further research.

5. Conclusion

Kelp forests are productive marine habitats known to facilitate many trophic and non-trophic interactions and a diverse associated fauna. Few studies focus on potential feedbacks this fauna may provide that benefit kelp directly and indirectly. Here we describe a mechanism in which fish may assist in dispersal of short-lived low motility kelp zoospores since an important component of the diet of *A. punctatus* is kelp and adult fish fed directly on kelp tissue reducing the epiphytic algal cover and leaving intact the internal cell layers of kelp. Further

zoospores remained viable after digestion and kelp sporophytes were produced at similar rates to undigested reproductive tissue. Although further research is required to comprehend the benefits provided by this herbivorous fish to kelp demography, we provided evidence of potential positive links between species that co-occur in temperate environments.

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Conflicts of interest

None.

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