ORIGINAL PAPER

Highly seasonal reproduction in deep-water emergent *Desmophyllum dianthus* (Scleractinia: Caryophylliidae) from the Northern Patagonian Fjords

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Abstract

Desmophyllum dianthus is a cosmopolitan species usually found at 1000–2500 m depth in the deep ocean, but in the Patagonian fjords can be found in shallow waters up to 8 m due to deep-water emergence. The purpose of this study was to determine the reproductive biology and seasonality of the scleractinian cup coral *D. dianthus* from the Chilean fjord region using histological techniques. Corals were collected via SCUBA approximately every 3 months from August 2012 to September 2013 from three sites—Lilihuapi ($n=76$) and Punta Huinay ($n=59$) in the Comau Fjord; and Morro Gonzalo ($n=44$) in the Reñihué Fjord (42.0°S–42.35°S). This study determined that *D. dianthus* is dioecious, and reproduction is highly seasonal, spawning at the end of austral winter (August) and beginning gamete production in early spring (September). Gametogenesis tracks with known fjord primary productivity and the fjords were coolest and most saline in August, potentially cueing spawning. Owing to the presence of late-stage oocytes in August 2012 and the absence of larvae, we hypothesize that *D. dianthus's* mode of reproduction is broadcast spawning. Oogenesis starts in September with previtellogenic oocytes (25–200 μm) that slowly develop into vitellogenic oocytes (200–380 μm) by June. Fecundity is high compared to other deep-sea scleractinians, ranging from 2448 ($\pm$ 5.13 SE) to 172,328 ($\pm$ 103.67 SE) potential oocytes per polyp. This research provides the first insight into *Desmophyllum dianthus's* reproductive biology and yields an important baseline for continuing work on this benthic habitat builder.

Introduction

Desmophyllum dianthus (Esper 1794) is a cosmopolitan, solitary, deep-water scleractinian that is a slow-growing (0.5–3.6 mm year^{-1}), long-lived (~ 200 year) species of cold-water coral and often lives on steep, rocky ledges or

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overhangs between 1000 and 2500 m (Cairns 1995; Försterra and Häussermann 2003; Adkins et al. 2004; Addamo et al. 2012; Jantzen et al. 2013). Cold-water corals are a diverse group of Anthozoans usually inhabiting continental shelves, seamounts, and deep-sea ridges (Roberts 2009). Cold-water corals are generally azooxanthellate, meaning they do not harbor photosynthetic algal symbionts, but feed on zooplankton and detritus. In contrast to tropical coral, cold-water corals do not need sunlight to obtain energy, which enables them to live below the photic zone (Keller 1976; Freiwald et al. 2004), though many species live outside these boundaries (Cairns 2007; Waller et al. 2014). Cold-water corals are often important ecosystem engineers that provide vital habitat to a variety of species, including invertebrates and commercially important fishes and crustaceans (Lawrence and Herrera 2000; Roberts and Hirshfield 2004; Robert et al. 2006; Brancato et al. 2007; Stone and Shotwell 2007; Braga-Henriques et al. 2013). In their shallower depth range, they are also described as a habitat-forming species in Chilean Patagonia (Försterra et al. 2017).

Despite their diversity and their important role in benthic deep-sea communities, the biology and ecology of

cold-water corals are not well understood, especially in comparison to their shallow-water counterparts (Roberts 2009), primarily because of the logistical difficulties in studying deep-sea species. Collections are complex, expensive, and often only yield small samples sizes. Furthermore, sampling in the same place twice is rare, making ecological studies sparse.

In the northern part of Chilean Patagonia, *D. dianthus* specimens live as shallow as 8 m (Försterra and Häussermann 2003). This phenomenon is known as deep-water emergence—when a deep-sea species inhabits depths shallower than their usual distribution due to bathyal or abyssal-like conditions (Waller et al. 2014). Deepwater emergence of cold-water coral species has only been documented at four other locations globally—Alaska (Stone and Shotwell 2007; Waller et al. 2014), New Zealand (Parker et al. 1997), Norway (Brooke and Järnegren 2013), and Sweden (Wisshak et al. 2005). It is hypothesized that these coral species occur at such shallow depths due to cold-water temperatures and high oxygen content within the fjords (Waller et al. 2014). In the three northernmost Chilean fjords, *D. dianthus* corals form extensive banks below 20 m, which provide habitat for numerous associated species (Försterra et al. 2017). Here *D. dianthus* populations reach densities of 1500 individuals m^{-2} (Försterra and Häussermann 2003; Häussermann and Förstera 2009). In addition, these corals grow along a pH range between 7.4 and 8.1, living close or even below the aragonite saturation horizon (Jantzen et al. 2013).

Reproduction is a fundamental ecological process, vital to the propagation and success of a species over time. Of the 3000 + known species of cold-water corals, information on reproductive biology is known for fewer than 60 species (Feehan and Waller 2015; Rakka et al. 2017; Rossin et al. 2017). Reduced reproductive energy allocation can indicate environmental stress (Van Veghel and Bak 1994; Richmond 1997; Zakai et al. 2000; Harrison and Ward 2001; Bongiorni et al. 2003; Waller and Tyler 2005), as reproduction, and somatic growth are two key life processes that require energy within an organism. When stresses occur, such as disease, temperature change, loss/reduction of food source, or low oxygen content, energy is diverted away from one or both of these areas to compensate. This relationship has been experimentally demonstrated in numerous species, across multiple phyla, from echinoderms to fishes (Pankhurst and Van Der Kraak 1997; Lawrence and Herrera 2000), making them useful proxies for examining populations under stress.

The Patagonian fjords are some of the least studied marine environments in the world, in spite of the dynamic and diverse ecosystem they support (Häussermann and Förstera 2009). Only minimal oceanographic and biological frameworks have been established, and marine manage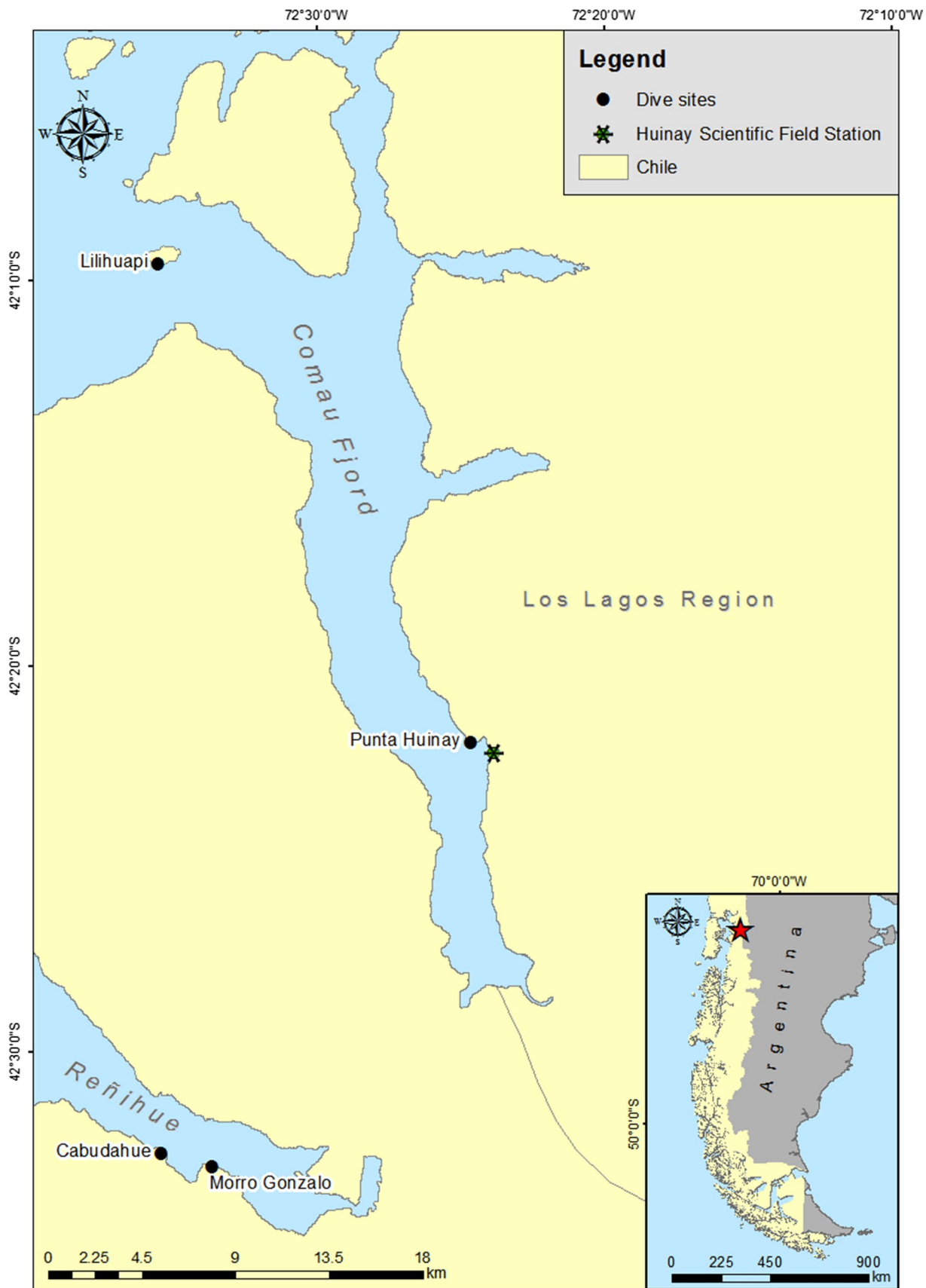ment plans are limited, unsustainable, and difficult to enforce (Buschmann et al. 2009; Niklitschek et al. 2013). Within the past two decades, anthropogenic impacts such as finfish aquaculture and infrastructure projects have substantially increased within this region (Försterra et al. 2017) and Chile is now the second largest producer of farm-raised salmon globally (Niklitschek et al. 2013). The Patagonian fjords represent one of the world's largest fjord systems, with a coastline of more than 90,000 km, with high biodiversity, high productivity, and home to several deep-water emergent species (Häussermann and Förstera 2009). Ensuring the sustainable management of the Chilean fjord region and their resources will require detailed biological information on both the benthic and pelagic species present within this system.

Deep-water emergence within the Patagonian fjords presents a unique opportunity to develop an ecological time series on an ordinarily deep-sea species. *Desmophyllum dianthus*, a solitary scleractinian, is typically found at over 200 m depth, but large accumulations are found at depths that are accessible by SCUBA in the northernmost fjords (Häussermann and Förstera 2009). This allows repetitive sampling from a single population and thus enables ecological studies. These shallow coral populations are windows into the deep ocean, providing a rare opportunity for in situ studies. An ecological study to understand the biology, reproduction and seasonality of *D. dianthus* is not only important for increasing our understanding of cold-water coral ecology in general, but also important for understanding a major benthic species found throughout the Chilean fjord region, and the global deep ocean.

The purpose of this study was to investigate the reproductive biology and seasonality of the cold-water coral *Desmophyllum dianthus* in the shallow water of the northernmost fjords of Chilean Patagonia. The objectives of this study were to determine the sexuality, reproductive mode, oocyte size, fecundity, and seasonality of *D. dianthus* using histological techniques. Reproductive output (fecundity) was also compared between three sites.

Materials and methods

Whole *Desmophyllum dianthus* individuals (as this species is solitary and thus just a single polyp) were collected via SCUBA approximately every 3 months from August 2012 to September 2013 from three sites within the northern Patagonian fjords—Lilihuapi ($n = 76$) and Punta Huinay ($n = 59$) in the Comau Fjord; and Morro Gonzalo ($n = 44$) in Reñihué Fjord (Fig. 1; Table 1). In the Comau fjord, the maximum

Fig. 1 Map of study sites in the Comau and Reñihué Fjord, Southern Chile. *Desmophyllum dianthus* individuals were collected at these sites between August 2012 and September 2013 ▶

Table 1 Summary of collections locations, dates, depth and number of males, females and non-reproductive individuals

Location	Latitude	Longitude	Date	Depth (m)	F	M	NR
Punta Huinay	42°22′27″S	72°25′42″ W	15-Aug-12	25	0	0	3
Punta Huinay	42°22′27″S	72°25′42″W	17-Nov-12	25	0	0	11
Punta Huinay	42°22′27″S	72°25′42″W	1-Apr-13	25	0	0	1
Punta Huinay	42°22′27″S	72°25′42″W	7-Apr-13	25	1	6	8
Punta Huinay	42°22′27″S	72°25′42″W	13-Jun-13	25	2	3	5
Punta Huinay	42°22′27″S	72°25′42″W	21-Sep-13	25	0	1	14
Lilihuapi	42°9′44″S	72°35′54″W	10-Aug-12	19.5	1	1	10
Lilihuapi	42°9′44″S	72°35′54″W	11-Aug-12	19.5	1	0	2
Lilihuapi	42°9′44″S	72°35′54″W	17-Nov-12	19.5	1	0	10
Lilihuapi	42°9′44″S	72°35′54″W	24-Feb-13	19.5	7	4	6
Lilihuapi	42°9′44″S	72°35′54″W	13-Jun-13	19.5	3	8	1
Lilihuapi	42°9′44″S	72°35′54″W	22-Sep-13	19.5	1	0	23
Morro Gonzalo	42°32′46″S	72°37′7″W	13-Aug-12	27	0	5	10
Morro Gonzalo	42°32′46″S	72°37′7″W	21-Sep-12	27	0	0	9
Morro Gonzalo	42°32′46″S	72°37′7″W	19-Mar-13	27	3	7	5
Morro Gonzalo	42°32′46″S	72°37′7″W	20-Sep-13	27	0	0	6
Cabudahue	42°33′11″S	72°35′20″W	13-Aug-12	18.3	2	1	14

F female, *M* male, *NR* indicates number of non-reproductive individuals

tidal amplitude is 7.5 m and low-salinity layer (LSL) ranges from 0.5 m in the summer to 10 m thick in the winter with salinities varying between 0.2 and 10 PSU (Schwabe et al. 2006). In the Reñihué Fjord, LSL is unknown and tidal amplitude is estimated to be approximately 5 m (Häussermann per. obs.). Owing to the large distance of Morro Gonzalo from Huinay Scientific Field Station, samples were only collected on three occasions from this site throughout the 14-month study. Onset Hobo temperature (U24-002-C) and light (UA-002-64) loggers were deployed at all three sites (Lilihuapi 19.5 m; Punta Huinay 25 m; Morro Gonzalo 27 m) to collect temperature, salinity (every 60 min), and light (illuminance—every 30 min) data throughout the entire study as potential indicators of reproductive seasonality. Coral samples were also collected from a fourth site, Cabudahué ($n = 17$), on the first field expedition to the Reñihué fjord; however, the living corals at this site were deemed too small for a reproductive study, thus no environmental data loggers were deployed. The Huinay Scientific Field Station was the base camp for the field portion of this study. All samples were fixed in 10% formalin with a borax buffer and shipped to the Ira C. Darling Marine Center at the University of Maine for further analysis.

Upon arriving at the Ira C. Darling Marine Center each sample was assigned a random identification number to prevent the biasing of the results. The height, length, and width of all individuals were measured prior to histological analysis (Online Resource 1). In attempts to create a proxy for live coral tissue (as *D. dianthus* tissue does not extend all the way down the full height of the coral) polyp area (length × width) was used instead of height, length, and width. Each

coral was decalcified in Rapid Bone Decalcifer (64,143-40, Electron Microscopy Sciences) to remove their skeletons. Corals were then placed in Bouin's solution to complete decalcification, improve staining and firm up tissue.

After 36 h in Bouin's solution, all corals were transferred to 30% ethanol solution. Corals were then serially dehydrated by 10% ethanol increments for 30 min each. At 70% ethanol, three–five mesenteries were dissected out of each polyp and put into a labeled histology basket. Batches of 15 or 20 baskets were then serially dehydrated from 70 to 100% ethanol by 10% ethanol increments (with the exception of including 95% ethanol between the 90 and 100%) for 30 min. Baskets were left in 100% ethanol overnight (~ 10 h). The following morning baskets were cleared in three 30-min submersions of toluene. After the final toluene submersion, baskets were transferred into melted paraffin wax (Paraplast Plus, Fisher Scientific) within an oven set at 60 °C. Baskets went through three wax changes over a 48-h time period.

Prepared coral mesenteries were then embedded into paraffin wax blocks. All blocks were sectioned with a Leica Microtome (RM2155) leaving 45 μm between sections (width of *D. dianthus* oocyte nucleus, to prevent double counting/measuring of oocytes) and slicing 5 μm sections. Sections were then mounted on glass slides and stained in Masson's trichrome.

Histological slides were examined using an Olympus (CX31) compound microscope with a Motic video camera attachment. Male and female individuals were identified (Table 1), and male spermatocysts and female oocytes were staged. Spermatocysts were categorized into four stages of maturity, I through IV (increasing in maturity) (Fig. 2).
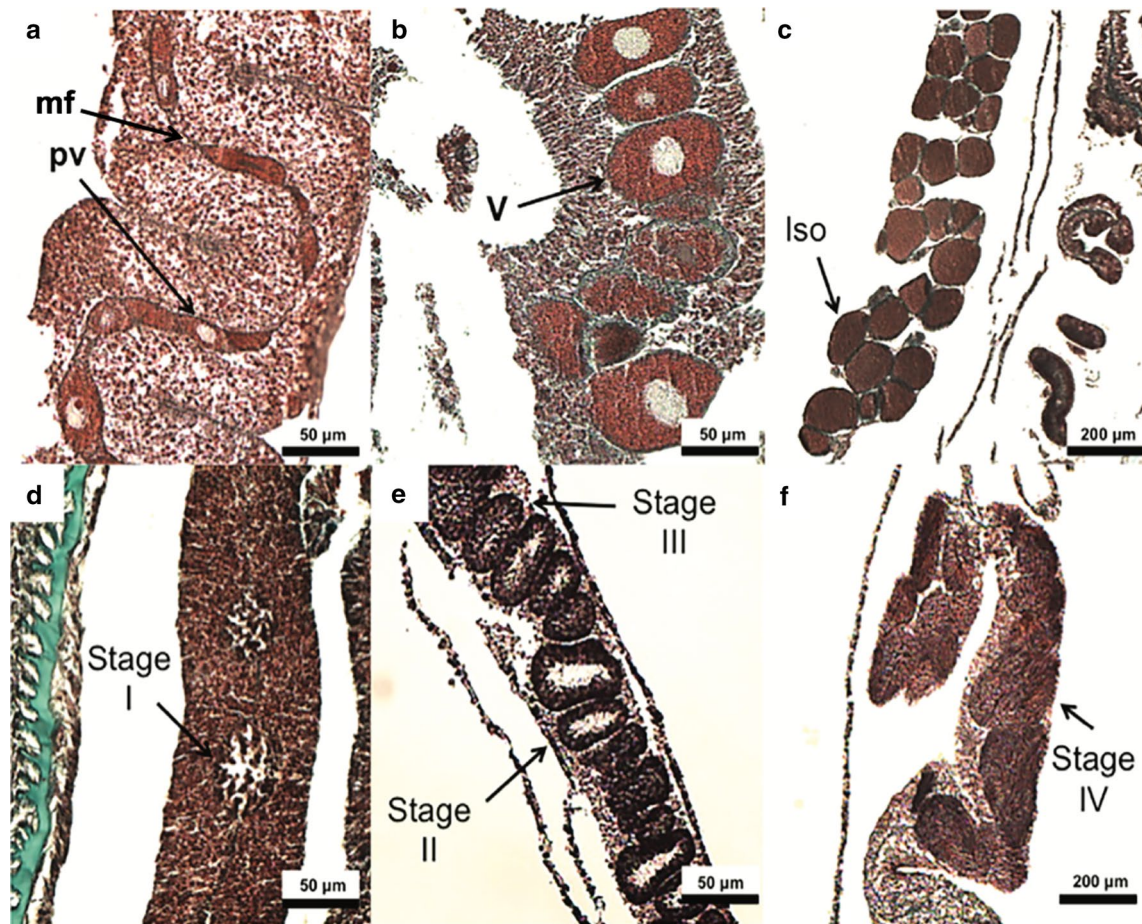
Fig. 2 Histological sections of *Desmophyllum dianthus* reproductive anatomy. **a** Female showing previtellogenic oocytes (pv) and the mesenterial filament (mf), **b** female showing vitellogenic oocyte (V), **c** late-stage oocytes with no nucleus (undergoing meiosis) (lso), **d–f** spermatogenesis: males showing spermatocyst stages, from least mature (stage I) to mature (stage IV)

Oocytes were sorted into two stages: vitellogenic and previtellogenic (Fig. 2). One hundred random spermatocysts were counted and staged for each male individual; spermatocyst frequency diagrams were constructed from these results. For each female individual, one hundred random oocytes were imaged and then analyzed using ImageJ software (NIH) to calculate oocyte diameter (a "Feret" diameter function was used to determine the area of the oocyte as if it were a perfect circle). Oocyte size frequency diagrams were constructed from the results.

Total fecundity was calculated by counting all previtellogenic and vitellogenic oocytes (that had a nucleus present) within the three mesenteries. For the purpose of this study, potential fecundity was defined as an individual's maximum potential reproductive output (number of oocytes per polyp). To determine average potential fecundity the total number of oocytes was quantified in three mesenteries, averaged, then multiplied by the total number of mesenteries (96: Zibrowius 1980).

Seasonality was determined by a nonparametric Each Pairs Wilcoxon test comparing stages of spermatocyst growth in males throughout the study period and average oocyte size frequencies and average fecundities between months in females. Linear regressions were used to determine relationships between coral height, length, width, and fecundity. An additional Each Pairs Wilcoxon test was used to compare coral dimensions (height, length, and width) between locations.

Results

Environmental analysis

Lilihuapi

Out of the 14-month environmental observation period, at Lilihuapi, the highest average monthly temperature was observed in February 2013 (13.05 °C) (Fig. 3a). The lowest
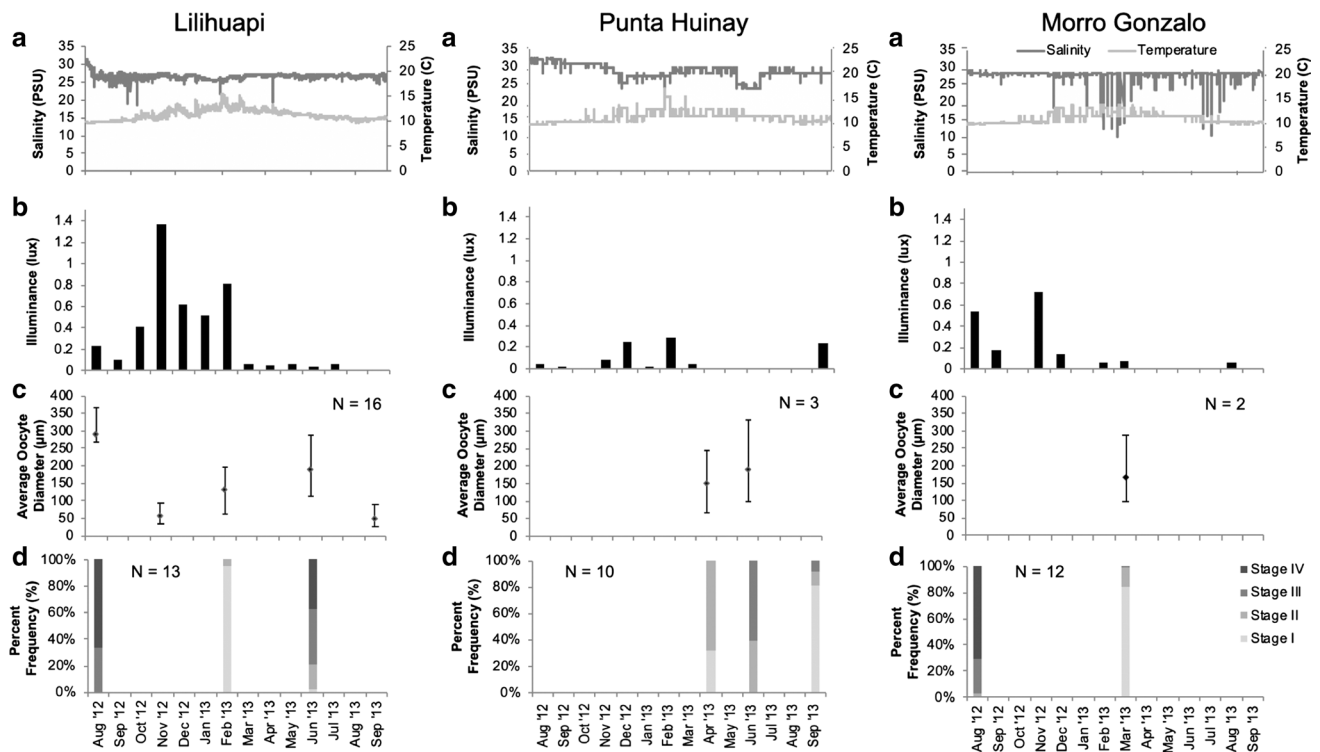
Fig. 3 Seasonality at Lilihuapi, Punta Huinay and Morro Gonzalo in terms of environmental and reproductive data from August 2012 to September 2013. **a** Salinity and temperature at Lilihuapi (left), Punta Huinay (center), and Morro Gonzalo (right). **b** Average light intensity per month from August 2012 to July 2013. **c** Average Oocyte diam-eter per month: Lilihuapi, Punta Huinay, and Morro Gonzalo. Error bars indicate standard error. **d** Percent frequency of spermatocyst stages per month at Lilihuapi, Punta Huinay, Morro Gonzalo (*N* number of individual corals used)

average monthly temperature was in August 2012 (9.79 °C). The general temperature trends were warming from September to April and cooling from May to August. Salinity was lowest in January 2013 (26.1 PSU) and highest in August 2012 (28.17 PSU) (Fig. 3a). The light intensity logger only recorded from August 2012 to July 2013 due to sensor failure. During that 12-month period light intensity was greatest in November 2012 (1.37 lx) and lowest in June 2013 (0.03 lx) (Fig. 3b).

Punta Huinay

The highest average monthly temperature at Punta Huinay during the 14-month environmental observational period was in February 2013 (12.34 °C) (Fig. 3a). The lowest average monthly temperature was in August 2012 (9.96 °C). The general temperature trends were warming from September to February and cooling from March to August. Salinity was lowest in June 2013 (25.73 PSU) and highest in August 2012 (31.50 PSU) (Fig. 3a). Light intensity (illuminance) was greatest in February 2013 (0.28 lx). The lowest average illuminance occurred in October 2012, and April through August (0.0 lx; Fig. 3b). It is important to note that a large

sea star had to be removed from covering the light sensor on three separate occasions, which undoubtedly affected light intensity readings.

Morro Gonzalo

Concordant with the previous two collection locations, Morro Gonzalo's highest average monthly temperature during the study was observed in February 2013 (12.35 °C) (Fig. 3a). The lowest average monthly temperature was in August 2012 (9.31 °C). The general temperature trends were warming from September to February and cooling from March to August. Salinity was lowest in March 2013 (26.62 PSU) and highest in August 2012 (28.23 PSU) (Fig. 3a). Light intensity was greatest in November 2012 (0.72 lx) and lowest in May and June 2013 with a monthly average of 0.0 lx (Fig. 3b).

Gametogenesis

Desmophyllum dianthus is a gonochoric species, having separate male and female individuals. However, the sex of an individual is not apparent from its outward appearance and can only be determined by histological analysis. No

Fig. 4 Percent frequency of oocyte diameter per month from females collected in the Comau fjord (Lilihuapi and Punta Hui-nay collection sites). Error bars represent standard error. Black arrows indicate median oocyte diameter (μm). *N* number of individual corals used
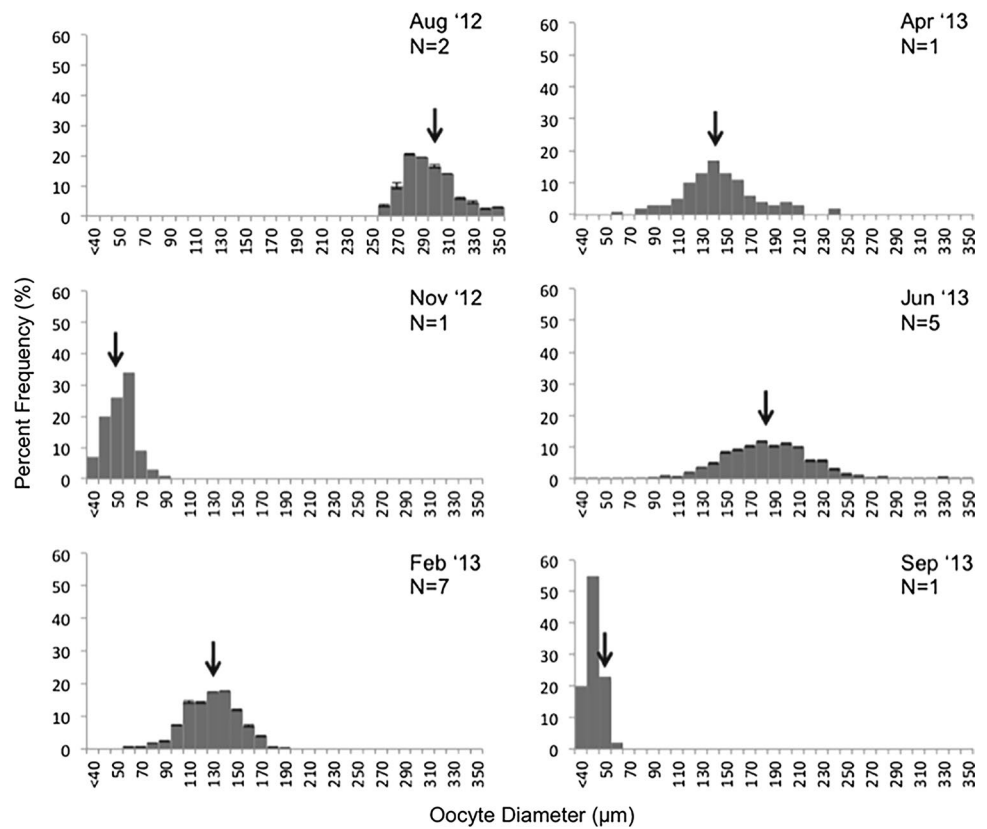
hermaphrodites were found in this study. Out of 195 corals collected, 36 were males, 22 were females, and the remaining 137 were non-reproductive. *Desmophyllum dianthus* in this area has a sex ratio of approximately 2:1 male to female. This study found that *D. dianthus* had spermatocysts and ooyctes embedded in the mesenteries. Spermatogonia and oogonia originate from mesenterial filaments (Fig. 2a). Previtellogenic oocytes ranged from 25 to 200 μm in diameter while vitellogenic oocytes ranged from 200 to 380 μm (Fig. 2a, b). Two female's oocytes were undergoing meiosis when collected in August 2012 (Fig. 2c, termed late-stage oocytes). Stage one spermatocysts were small (average diameter: 108.79 μm ± 5.38 SE), usually standing alone, and were surrounded by the mesenterial filament with patchy spermatids within (Fig. 2d). Stage two spermatocysts had a distinct lumen with a thick border of densely packed developing sperm and were 234.89 μm (± 20.61 SE) in diameter (Fig. 2e). Stage three spermatocysts also had a thick border of densely packed spermatids but the lumen was filling with lightly or densely packed sperm. Stage three spermatocyts were 267.93 μm (± 18.58 SE) in diameter (Fig. 2f). Both stage two and three spermatocysts were usually closely packed together next to other spermatocysts. Stage four spermatocysts were large (average diameter: 271.07 μm ± 13.26 SE) with dense mature sperm and visible sperm tails (Fig. 2f).

In the Comau fjord females had high frequencies of pre-vitellogenic oocytes in November, February, and April 2012 as well as September 2013 (Fig. 4 and Online Resource 2). In June 2013 the Comau females had approximately equal frequencies of previtellogenic and vitellogenic oocytes. In August 2012 all females had only vitellogenic oocytes present within the mesenteries (Fig. 4). Females collected in September 2013 had the smallest average oocyte diameter (50.96 μm ± 1.19 SE) while August 2012 females had the greatest average oocyte diameter (301.25 μm ± 1.06 SE) (Fig. 4). The general trend for females in the Comau fjord was small previtellogenic oocytes beginning to develop in September, slowly growing in size and yolk content before transitioning to vitellogenic oocytes in June. Oocytes developed uniformly and steadily, at no point in this study were small previtellogenic oocytes and large vitellogenic oocytes observed together in a single sample. Differences in mean monthly oocyte diameters at the Lilihuapi location were highly statistically significant ($P < 0.0001$ for all comparisons with the exception of September 2013 vs. November 2013 having a $P = 0.0003$), confirming that *D. dianthus* is highly seasonal. In the Reñihué fjord, female samples were only collected in March 2013, thus no overall trend can be reported. However, average oocyte diameter of Reñihué females in March follows the general seasonal trend seen in the Comau fjord (Fig. 3).
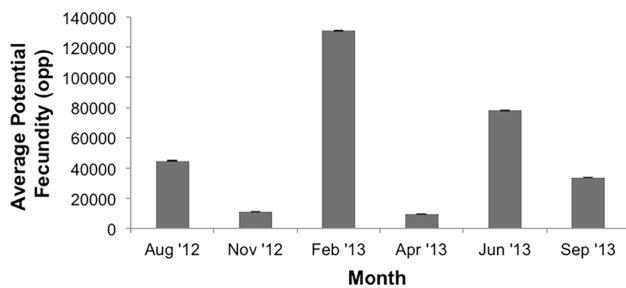
Fig. 5 Average potential fecundity by month at Lilihuapi. Error Bars indicate standard error. August 2012 $N = 2$, $n = 3567$; November 2012 $N = 1$, $n = 414$; February 2013 $N = 7$, $n = 26,756$; April 2013 $N = 1$, $n = 366$; June 2013 $N = 5$, $n = 9012$; September 2013 $N = 1$, $n = 1302$ (N number of individual corals used; n number of oocytes counted)

Fecundity

Females from Lilihuapi in February 2013 had the highest average potential fecundity at 172,328 (± 103.67 SE) oocytes per polyp (opp). The lowest average potential fecundity was in August 2012 at Cabudahué in the Reñihué fjord: 2448 (± 5.13 SE). Fecundity peaked in February (Fig. 5). Fecundity increased by 1213.3% from November 2012 to February 2013. From February to June 2013 fecundity decreased by 64.5%. The standard error of the number of oocytes between individuals is $\pm 18{,}714.9$. The number of oocytes varied by ± 71.6 SE within individuals. The average fecundity at Lilihuapi in June 2013 96107 (± 213 SE) opp is nearly ten times greater than the average fecundity at Punta Huinay that same month 10,944 (± 0.96 SE) opp.

Morphometrics

There was a significant difference in polyp size (height, length, and width) between the three collection locations (Fig. 6). There is a significant difference in height, length and width between Lilihuapi and Punta Huinay There was no significant difference in height, length, or width between Lilihuapi and Morro Gonzalo (Fig. 6). However, a significant difference was found in terms of height, length, and width between Morro Gonzalo and Punta Huinay (Fig. 6). In general Punta Huinay individuals were small while Lilihuapi and Morro Gonzalo individuals were large and dense. No significant trend was found between fecundity and polyp area at Lilihuapi in February or June 2013 ($R^2 = 0.164,418$ and $R^2 = 0.29351$, respectively). Nor was a trend found between polyp area and fecundity at Morro Gonzalo in the Reñihué fjord ($R^2 = 0.0072$). Size of first reproduction in males was: height: 43.1 mm, length 10.5 mm, width 7.8 mm and area: 81.9 mm^2. The size of first reproduction in females was: height: 33 mm, length: 13 mm, width: 11.5 mm, and area: 149.5 mm^2.
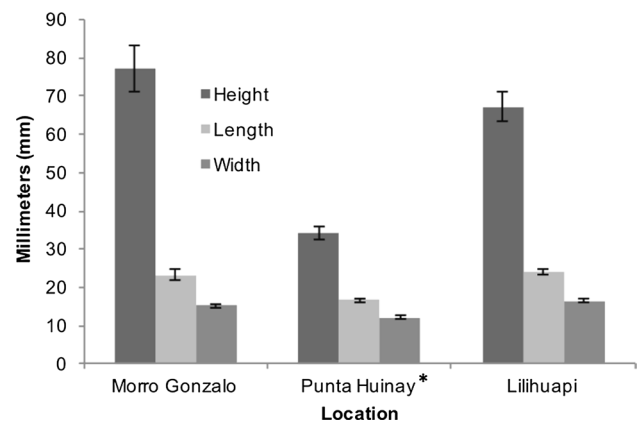
Fig. 6 Size of collected *Desmophyllum dianthus* individuals by location. Error bars indicate standard error. Morro Gonzalo and Lilihuapi are not statistically different from one another (height: $P = 0.20408$, length $P = 0.48006$ and width $P = 0.36812$). Punta Hunay (marked with astersik) is statistically different from both Morro Gonzalo (height: $P = 0.0001$, length $P = 0.0001$ and width $P = 0.0001$) and Lilihuapi (height: $P = 0.0001$, length $P = 0.0001$ and width $P = 0.0001$). Individuals measured—Morro Gonzalo $N = 44$; Punta Huinay $N = 55$; Lilihuapi $N = 74$ (N number of individual corals used)

Discussion

The purposes of this study were to describe the sex, sex ratio, reproductive mode, and seasonality of *Desmophyllum dianthus*. *Desmophyllum dianthus* is gonochoristic; none of the corals sampled were observed to have both male and female gametes. Gonochorism is common in cold-water scleractinians (Waller 2005; Brooke and Järnegren 2013; Feehan and Waller 2015), in contrast to tropical shallow-water scleractinians, which tend to be hermaphroditic (Baird et al. 2009). Gonochorism is postulated to be an ancestral trait in Scleractinia (Harrison 2011), there is strong support that cold-water azooxanthellate corals may have given rise to zooxanthellate corals (Veron 1995).

The primary mode of reproduction in all scleractinians (zooxanthellate and azooxanthellate) is broadcast spawning (Harrison and Wallace 1990). It is likely *D. dianthus* is a broadcast spawner as no brooded larvae have been observed during numerous dives since 2003, in hundreds of photos taken of the species, or in the histological preparations of this study (Häussermann per. obs.). Further, vitellogenic oocyte sizes (200–380 μm) suggest pelagic or demersal development of larvae (Strathmann 1987). Strathmann (1987) defines parameters for cnidarian larval development based on maximum oocyte sizes: 100–250 μm is an indicator of planktotrophic development while 500–800 μm suggests lecithotrophic development. The maximum oocyte size (380 μm) in *D. dianthus* falls between the two ranges making speculation on the mode of larval development difficult. Recent literature further blurs the lines between maximum

oocyte size that indicates one type of larval development versus another. *Caryophyllia cornuformis* (max oocyte size 340 µm) and *Enallopsammia rostrata* (max oocyte size 400 µm), both cold-water scleractinians, have oocyte sizes comparable to *D. dianthus* yet both are suggested to have lecithtrophic larvae (Burgess and Babcock 2005; Waller et al. 2005). In addition, *Lophelia pertusa's* maximum oocytes size ranges from 140 to 337 µm has been confirmed via in situ experimentation to have lecithotrophic larvae (Brooke and Järnegren 2013) in addition, *Solenosmilia variablilis* is suggested to have lecithotrophic larvae despite its small maximum oocyte size of 242 µm (Pires et al. 2014). By contrast, *Caryophyllia sequenzae* is suggested to have planktotrophic larval development despite a maximum oocyte size of 450 µm (Waller et al. 2005). In the absence of observations of larvae or larval behavior it is impossible to determine whether *D. dianthus* has feeding or non-feeding larval development, however, increased food availability at spawning times (discussed below) may indicate a possible planktotrophic larval habit.

Oocytes were observed from early oogenesis in September to late-stage vitellogenesis in August of the following year. Germinal vesicle breaking down in late-stage oocytes indicates fertilization in mid-August 2012, although it is unknown whether fertilization took place inside or outside of the polyp. Additionally, based on the absence of planula larvae in any of the histological sections and the onset of oogenesis in September it is highly likely that *D. dianthus* uses broadcast spawning as its mode of reproduction. The high proportion of non-reproductive individuals is likely due to the time of sampling. The majority of non-reproductive individuals were from August and September. It is possible that mature individuals already released their gametes in August and mature individuals in September had yet to begin the next cycle of gametogenesis.

Reproduction in *Desmophyllum dianthus* is highly seasonal. Based on the combined data of male individuals spermatogenesis took 11 months, although, in some samples, spermatocytes matured sooner and stayed mature until speculated release in August. This is not uncommon in invertebrates, as spermatogenesis is generally less energetically expensive than oogenesis (Goffredo et al. 2002; Baillon et al. 2011). Oogenesis also took approximately 11 months: gametes formed in September and germinal vesicle break down (and likely spawning) occurs in late-stage oocytes the following August, at the end of the Austral winter.

Many corals utilize a timed gamete release triggered by environmental cues (such as temperature, lunar cycle, food availability, etc.) to increase fertilization success. Spawning events are commonly observed in shallow water species (Babcock et al. 1986; Richmond and Hunter 1990; Richmond 1997; Mangubhai and Harrison 2008), but substantially less is known about cold-water coral spawning events due to difficulties surrounding their observation and collection. The data collected in this study points to a spawning event in August for shallow populations if *D. dianthus* in the Chilean fjords. Determining what factor, or factors, cues *D. dianthus* reproduction is challenging because of the dynamic environment. Within the speculated timeline of *D. dianthus* spawning event(s), there is an increase in food availability with the spring bloom beginning in September. Additionally, water temperature falls to an annual minimum within days of the observed germinal vesical breakdown in oocytes and waters become most saline in August. It is likely food availability is the largest factor influencing the timing and phases of gametic development while environmental variables such as temperature or salinity ultimately cue spawning events.

Timing of food fall (particulate organic matter) has been speculated to trigger various aspects of deep-sea invertebrate reproduction due to the increase in energy availability (Tyler et al. 1992;,1993; Eckelbarger and Watling 1995; Gooday 2002; Young 2003; Mercier and Hamel 2009). Some cold-water corals reproduce seasonally and may also be cued by seasonal food fluxes to the benthos or other environmental factors (Waller and Tyler 2005; Mercier et al. 2011). The reproductive cycle of *D. dianthus* closely matches the timing of high primary productivity cycle within the Chilean fjords, with spawning at the beginning of the spring bloom (September/October) (Iriarte et al. 2007), and may indeed be triggered by the initial algal bloom of *Skeletonema* spp. (Montero et al. 2017a). This bloom marks the start of a period of relatively high primary productivity from late August to May in the Patagonia fjords that likely places *D. dianthus* larvae (if they have planktotrophic development) and/or new recruits at an advantage for growth, development, and successful recruitment because of the increased food availability at this time (Montero et al. 2017a). Development of spermatogonia and oogonia also closely tracks the primary productivity cycle, with gametogenesis starting after the influx of food from the spring bloom (a time when the corals would be ready to start producing another cohort, because of the spawning event in late August). Vitellogenesis may also be triggered by the fall bloom (April/May), and a deep winter bloom of dinoflagellate *Heterocapsa triquetra* may allow *D. dianthus* to increase lipid stores during June, July, and August in preparation for spawning (Montero et al. 2017b).

In this study, other external cues that could be triggering gamete release are temperature and salinity. At all locations, the water was coolest and most saline in August 2012 and 2013, in agreement with previously reported temperature seasonality in this region (Laudien et al. 2012a, b, 2013, 2015). In general, water temperatures in the Comau fjord peak in February and fall to a minimum in August (Laudien et al. 2012a, b, 2013, 2015). Interestingly, at the Lilihuapi site germinal vesicle breakdown in oocytes occurred

48–72 h prior to water temperature reaching the annual minimum (9.51 °C) on August 13th, 2012. In tropical zooxanthellate scleractinians, temperature is commonly known to cue gamete release (Babcock et al. 1986; Harrison and Wallace 1990), so this has potential as a spawning cue in our study species.

The reproductive cycle of another cold-water coral found in this same area in the Chilean fjords, *Primnoella chileansis*, follows an almost identical cycle, with reproduction tracking with fjord primary productivity (Rossin et al. 2017), indicating that both of these benthic species likely follow the environmental conditions of the fjord closely with respect to reproductive processes. These data suggest that in these shallow populations of *D. dianthus*, gametogenesis is triggered with increased food availability in September then tracks fjord primary productivity to produce mature sperm and oocytes by austral winter. Annual low temperature and/or high salinity may then cue spawning events in *D. dianthus*.

In studies published to date, the maximum oocyte diameters of deep-sea scleractinian species range from 100 to 5167 µm (Brooke and Young 2003; Waller et al. 2008). *Desmophyllum dianthus* maximum oocyte diameter (380 µm) is relatively small compared to other deep-sea scleractinians (Brook and Young 2003; Burgess and Babcock 2005; Waller 2005, Waller et al. 2008; Goffredo et al. 2010; Pires et al. 2014), especially considering this population's proximity to the Antarctic, where known scleractinians' oocyte sizes range from 4800 to 5167 µm (Waller et al. 2008). Although Antarctic scleractinians have larger egg sizes, their fecundity is lower, likely as compensation for the extra energy involved in producing larger oocytes. A weak correlation was found between fecundity and polyp area in this species. Two other deep-sea scleractinians, *Lophelia pertusa* and *Madrepora oculata* from the Northeast Atlantic, were also found to have no statistically significant trend between fecundity and size (Waller and Tyler 2005).

In this study, the size of first reproduction is a polyp area of 82 mm^2 in males (coral height: 43.1 mm) and 150 mm^2 in females (coral height: 35.8 mm). It is possible that females become reproductive somewhere between a polyp area of 82 and 150 mm^2; however, this study found no reproductive females smaller than 150 mm^2. It is also important to note that *D. dianthus* is relatively slow-growing, averaging a growth rate of 2.3 mm year^{-1} within the fjords (Försterra and Häussermann 2003). In ideal circumstances with consistent growth (assuming growth is vertical), corals would have to reach a minimum height of 36 mm or an estimated 15.6 years of age before becoming reproductively mature.

Due to a low number of reproductive individuals, statistical analysis could not be used to determine differences in reproductive characteristics determined between collection sites, though it is important to note average potential fecundity was 10× lower in individuals collected from the Punta Huinay site than those collected from Lilihuapi in the same month (June). Curiously, *D. dianthus* individuals from the Punta Huinay collection site were statistically smaller (mean height: 34.45 mm; length: 16.67 mm; width: 12.19 mm) compared to those collected from the other two sites (Lilihuapi: Mean height: 67.28 mm; length: 24.11 mm; width: 16.48 mm. Morro Gonzalo mean height: 77.1 mm; length: 23.32 mm; width: 15.34 mm). At the Punta Huinay site, there are a variety of environmental factors that might explain the small sizes and decreased reproductive potential of *D. dianthus*. Punta Huinay is located near the Lloncochaigua river and receives seasonal freshwater input that impacts the salinity and pH of the site (Jantzen et al. 2013). Finfish farming could be an environmental stressor for a sessile benthic organism like *D. dianthus*, as sedimentation, such as that created from overfeeding and concentrated fish waste, can be a significant, even lethal stress to corals (Rogers 1983, 1990; Riegl and Branch 1995; Häussermann et al. 2013). Punta Huinay is 400 m from a salmon-farming facility, whereas the other two sites are not in close proximity to fish aquaculture. Hypoxia or anoxia after strong algal blooms, intensified by finfish aquaculture, may also be taking its toll on the benthic community at the Punta Huinay site (Försterra et al. 2014). Though it is unknown to what degree each of these variables may impact the growth and reproduction of *D. dianthus*, it seems likely that salmon farming could have an effect and needs to be evaluated further.

In summary, this study identified the sex, likely mode of reproduction, size of first reproduction, and fecundity for *Desmophyllum dianthus*, a cosmopolitan, cold-water scleractinian species. This contribution provides important biological data on an ecologically important coral species found in shallow and deep waters around the globe. For future research, the next step would be to compare the reproduction and seasonality of shallow populations and deep populations of *D. dianthus* found in the northern Patagonian fjords and worldwide. One could anticipate deeper global populations of *D. dianthus* to be less fecund, similar to what has been seen in *Fungiacyathus marenzelleri* (Waller et al. 2002; Flint et al. 2007; Waller and Feehan 2013) due to a reduction in food availability. Conversely, deeper depths may function as a refuge to *D. dianthus* populations. A study by Gori et al. (2016) found that temperature fluctuation significantly affected calcification rates in *D. dianthus* whereas decreased pH has less of an impact (Gori et al. 2016). With less fluctuation in temperature and greater distance from anthropogenic impacts, deeper populations within the Chilean fjords may be isolated from major environmental stressors (Fillinger and Richter 2013). Deep populations within the Chilean fjords may expend less energy compensating for stress and may, therefore, have more energy for reproduction and growth. Currently there is limited biological and physical data on the Patagonian fjord region, and it is important to

understand the biology of habitat-building organisms as well as effects of anthropogenic impact to this incredibly dynamic and diverse ecosystem.

Compliance with ethical standards

Conflict of interest This work was funded by National Geographic and the National Science Foundation (USA), and the authors declare there are no conflicts of interest.

Ethical approval All applicable international, national and/or institutional guidelines for sampling, care and experimental use of organisms were followed and all necessary approvals have been obtained.

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