This study provides the first data on the reproductive biology of the spiny gurnard Lepidotrigla dieuzeidei. A total of 1027 individuals were collected monthly from September 2011 to August 2012. The total length of the individuals in the sample ranged from 7.46 cm to 14.62 cm with a female to male ratio of 1 to 0.59. Positive allometric growth was observed for both females (b=3.07) and males (b=3.08). Variations of condition factor were similar in both sexes with mean values of 1.07 for females and 1.01 for males. The fish L. dieuzeidei had a long spawning period extending from November to July based on monthly values of gonadosomatic index. Histological analysis showed that this fish was multiple synchronous developments of ovaries with multiple spawning events. The ovaries were always represented with a group of primary oocytes and a diverse population of vitellogenic oocytes, each specially recruited during the final stages of maturation in uniform or uneven phases. Batch fecundity was estimated gravimetrically using the hydrated oocyte method and was estimated to be around 558 to 10,230 oocytes per female. L. dieuzeidei is an important by-catch species and data from this study represent a valuable contribution to stock management.

Key words: oogenesis; spermatogenesis; gonad maturation; fecundity; oocyte diameter; multiple spawning fishes

INTRODUCTION

 Fisheries of deeper Adriatic Sea waters are mainly executed at depths of 200-300 m and it represents the largest area of occurrence of demersal shared stocks in the Mediterranean. Many different fishing gears exploit the demersal communities but trawl net represents the main gear in terms of catch and fleet power (CADDY, 1993; SÁNCHEZ et al., 2007). However, trawling exploitation in the Adriatic Sea is directed at a restricted number of target species. The main target fish species for the demersal trawl fishery are European hake (Merluccius merluccius), red mullet (Mullus barbatus barbatus), breams (Pagellus spp.), whiting (Merlangius merlangus), anglerfish (Lophius spp.), flatfish (Solea solea, Scophthalmus maximus, Scophthalmus rhombus, Platichthys flesus).

Studies of the impact of fishing have mostly focused on the target and economically important species and little is known about the impact...
to non-target species that have none or small economic value and have been characterized as by-catch species. One of the frequent by-catch species is spiny gurnard \textit{Lepidotrigla dieuzeidei} Blanc & Hureau, 1973 from the family Triglidae. It is a small benthic species distributed in the eastern Atlantic and the western and middle Mediterranean Sea and it prefers sandy and muddy bottoms at a depth between 60 and 180 m (JARDAS, 1996). There are around 114 species of family Triglidae in the world and only 8 species divided into 5 genera exist in the Mediterranean and the Adriatic Sea. \textit{L. dieuzeidei} and large-scaled gurnard, \textit{L. cavillone} are the only representatives of the genus \textit{Lepidotrigla}. Despite the high abundance of gurnard species, little is known about the biology of \textit{L. dieuzeidei}, especially reproduction data. For the Adriatic Sea, only available data for this species is an elliptic analysis of otoliths (MONTANINI et al., 2010).

Previous studies in other parts of the Mediterranean are based on the first record with the morphometric and meristic description of this species (ERYILMAZ, 2002; BAÑÓN, 2004; DALYAN & ERYILMAZ, 2006). The weight-length relationship was investigated in the Mediterranean (TORRES et al., 2012; BAŞUSTA et al., 2013a) and in the south coast of Portugal (OLIM & BORGES, 2006) while the age and growth and relationship between total length and otoliths dimensions were analysed in the northeast Mediterranean (BAŞUSTA et al., 2013b; BAŞUSTA et al., 2017).

Considering the lack of crucial biological data for this species, the main objective of this study was to investigate the annual reproductive cycle of \textit{L. dieuzeidei} in the Adriatic Sea. Sex ratio, length at sexually maturity, gonadosomatic index, fecundity, gonad development and oocyte size were analysed to obtain insight into the spawning activity for this by-catch species. Knowledge of reproductive biology of a fish species is essential for stock assessment and effective fishery management.

**MATERIAL AND METHODS**

**Sampling site, procedure and measurements**

Samples were collected from south Croatian offshore waters between the city of Dubrovnik and the north point of Pelješac peninsula (Fig. 1). A total of 1027 individuals were collected monthly, from September 2011 to August 2012. Specimens were caught at depths between 170 and 230 m using bottom trawl net (2 hauls, each lasted for approximately 5 h) during commercial fishing activities (vessel engine power 60kw). The total length of the net was 45 m with a mesh size of 60 mm and cod-end 24 mm, measured knot to knot. After catching all specimens were put on ice and transported to the laboratory for further analysis. For each fish, total length (Lt) was measured using a simple calliper to the nearest 0.1 mm and weighed (W) to the nearest 0.1 g. Fishes were gutted, and gonads were removed and weighed (Wg) with three decimal accuracy. Sex was determined by macroscopic observation of the gonads (MACER, 1974). To test differences in relation to the expected ratio 1:1, the sex ratio was examined using Chi-square ($\chi^2$) test with a probability level of 0.05. Age was estimated by interpreting growth rings on otoliths from 200 individuals. After removal otoliths were cleaned in distilled water, immersed in glycerol and examined with stereomicroscope on a black background under reflected light. Some otoliths were polished with sandpaper (type P220 and P80) due to calcium accumulation. Age estimations were made by three readers if the readings did not coincide.

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**Fig. 1.** Location of commercial fishing from town Dubrovnik to the north point of Pelješac peninsula (line), southern Adriatic Sea.
the otolith was rejected. Transparent and opaque rings were counted, one transparent and one opaque ring was considered as one year.

**Data analysis**

Growth was analysed using von Bertalanffy growth equation: $L_t = L_i e^{k(t - T)}$, where $L$ is the asymptotic length, $k$ is the growth coefficient, and $T$ is the hypothetical age for $L_t=0$ (Tomlinson & Norman, 1961). To describe the length-weight relationship, allometric growth equation was used: $W = a L^b$ (Ricker, 1977), where $W$ is the total weight (g), $L$ is the total length (mm) and $a$ and $b$ are constants estimated by last square method. Fulton’s condition factor was calculated following Froese (2006): $K = W/L$, where $W$ is total body weight (g) and $L$ is the total length (mm).

The spawning period was established based on the monthly variation of the gonadosomatic index (GSI) and calculated as $GSI = 100 \times W_g/W$, where $W_g$ is gonad weight (g) and $W$ is the total weight (g) (Ricker, 1977). To estimate size at first sexual maturity, the data were fitted in the equation: $P = 1/(1 + e^{(a - b \times L)})$; where $P$ is the probability that individuals are sexually matured, $L$ is their length and $a$, $b$ coefficients. The length when 50% of analysed individuals were mature was calculated according to Sparre & Venema (1998) as $L_{50%} = a/b$.

For histological analysis 30 individuals, randomly selected, from both sexes were used monthly. After weighing, gonads were fixed in 8% buffered formalin. A small piece of tissue from the middle of the gonad was tested. After dehydration and clearing, tissue was embedded in paraffin, sectioned on a microtome (5 µm) and stained with hematoxylin and eosin dye. All histological sections of ovaries were classified to assess the oocyte developing phase according to Tyler & Sumpter (1996) and Brown-Peterson et al. (2011). Oocytes were categorized as primary growth oocyte (PG), cortical alveoli oocyte (CA), vitellogenetic oocyte (Vtg), germinal vesicle migration (GVM) and hydration (H). Atresia of oocytes was assessed according to Hunter & Macewicz (1985a). Type of ovarian development was determined according to Wallace and Selman (1981) and testes Takashima & Hibiy (1995). For quantitative analysis oocyte diameter was measured using an image analysis system (AxioVision Release 4.8. software). Male germ cells were classified as spermatagonia (Sg), spermatocytes (St), spermatids (Sd) and spermatooza (Sz) (Grier, 1981).

It should be noted that the selection of the ovaries used for fecundity estimation was based on gonad histological sections showing that postovulatory follicles were not present. Batch fecundity was estimated gravimetrically using the hydrated oocyte method, where only oocytes larger than 500 µm were counted (Hunter et al., 1985). For each selected ovarian sample, three subsamples of 0.01 g from the anterior, central and posterior region of the gonad lobe were taken for counting migratory nuclei and hydrated oocytes. Batch fecundity for each female was estimated as the mean value of three subsamples that have been calculated as the number of the migratory nuclei and hydrated oocytes per unit weight of the subsample and the total weight of the gonad. Regression analyses were carried out relating fecundity to total length ($L_t$), total weight ($W$) and gonad weight ($W_g$). The relationships fecundity-length, fecundity-weight and fecundity-gonad weight were express by equation $F = a$, where $F$ is the fecundity, $X$ either $L_t$, $W$ or $W_g$, $a$ is the constant and $b$ is the exponent (Bagenal, 1978).

**Statistical analysis**

Comparison between means was performed using ANOVA test with multiple comparison tests (Tukey and Duncan) to identify similarity groups. Relations between time-series was analysed using the Cross Correlation Function (CCF) while the statistical difference between time series was analysed using the chi-square goodness of fit. Analysis of similarity between equations such as the male and female length-weight relationships was performed using ANCOVA method. Equation fitting and all statistical analysis were performed using NCSS 11 software (NCSS 11 Statistical Software 2016).
RESULTS

Total length (TL) of all analysed individuals (n = 1027) ranged from 7.46 to 14.62 cm. Both females and males were most dominant in length classes from 10 to 12 cm. Out of 1027 analysed individuals, 642 (62.5%) were females and 385 (37.5%) males. The overall female to male ratio was 1:0.59 and chi-square analysis showed it was statistically different (χ² = 63.31; P<0.001) than the 1:1 ratio.

Age was successfully determined in 71.5% (N=143) individuals, otoliths from the remaining 28.5 % (N=57) individuals were rejected because of disagreement between readers or impossibility to read otolith. The sample consisted of 77 females and 66 males. The total length of females ranged from 7.46 to 14.14 cm (11.43±1.54 cm) and males from 7.64 to 14.62 cm (10.57±1.73 cm). In the analysed sample, the oldest individuals had 4 years, the most common ages were two (N=50) and three (N=51) with a total body length of 9.52 to 13.20 cm. Three-years-old females dominated in the population. The lengths-at-ages for length classes 7-14 cm were used to estimate the parameters of the von Bertalanffy growth equation. The estimated parameters for both males and females are given in Fig. 2. Based on the obtained growth coefficient, males growth (k = 2.51) faster than females (k = 2.43) but they had a smaller asymptotic length (=13.64 cm) then females (=13.97 cm).

The length-weight relationships were described by the equation: W = 0.009(TL^{3.07}), r^2 = 0.945 and W = 0.008(TL^{3.08}), r^2 = 0.957 for females and males, respectively. Obtain b value for both females (b=3.07) and males (b=3.08) showed positive allometric growth of this species (Fig. 3).

Fluctuations of condition factor were similar in both sexes. Values ranged from 0.961 to 1.092 with a mean value of 1.075±0.04 for females and 1.012±0.05 for males. Chi² goodness of fit showed that both time series show no statistical difference (P>0.05), qualitatively, males showed slightly lower values. Monthly variation of condition factor showed an improved condition between April and November and lower from December to March. In both females and males, larger individuals had higher values of condition factor.

The highest gonadal activity was recorded from November to July showing the extended spawning period of this species. Average maximum values of gonadosomatic index (GSI) were recorded in April for females (8.21 ± 2.69%) and in December for males (0.22 ± 0.25%), while minimum values were recorded in August for both, females (1.74 ± 2.05%) and males (0.08 ± 0.06%) (Fig. 4). Cross correlation function analysis between the two-time series showed no statistically significant interaction between sexes, the changes of male GSI did not affect or follow the changes in female GSI. The male gonads were significantly smaller than female,
the average weight of male gonads was 0.02 ± 0.01 g and females 0.96 ± 0.58 g. Cross correlation function analysis of GSI and condition factor showed that there was no significant difference ($P>0.05$) between them (Fig. 5).

The total length of females used for estimation of the first sexual maturity ranged from 7.46 to 14.14 cm and males from 7.64 to 14.62 cm. Estimated length of sexual maturity was at 8.99 cm for females (Fig. 6a) and 10.01 cm for males (Fig. 6b) which corresponds with ages 1 and 2.

Histological sections of ovaries showed the presence of oocytes in primary growth, cortical alveolar and vitellogenic stage throughout the year. September (Fig. 7a) and October (Fig. 7b) are characterized by the increase in number of vitellogenic oocytes. Increase gonadal activity is visible from November (Fig. 7c) to February (Fig. 7f) which coincided with the annual increasing of GSI values. During this period number of oocytes in germinal vesicle migration stage started to increase and remained high until June (Fig. 7j). During May, oocytes in germinal vesicle migration and in hydration stage were most dominant, showing that this month represented one of the highest spawning peaks for this species (Fig. 7h) in accordance also to GSI values. The number of these developing stages started to decreases during July (Fig. 7k) and especially in August (Fig. 7l) when number of
oocytes in primary growth stage increased and atretic oocytes were visible on sections. Post-ovulatory follicles were present in ovaries during January (Fig. 7e), February (Fig. 7f), March (Fig. 7g) and in the period from May (Fig. 7i) until August (Fig. 7l).

Throughout the whole year, primary growth oocytes with a diameter smaller than 100 µm and vitellogenic oocytes with diameter from 100 to 500 µm were present in ovaries. Oocytes in germinal vesicle migration (from 500 to 700 µm) and hydration (> 700 µm) stage were not present during September, while the prevalence of these stages occurred from February to June.

Cross correlation function analysis between the monthly average oocyte diameter and monthly GSI showed a positive relationship between the two-time series. Moreover, the analysis showed that the time difference between the increase or decrease of the average oocyte diameter and a similar change of GSI is 1 month (Fig. 8).
Only 40 mature females (total length from 9.83 to 13.93 cm) fitted the criteria for estimating batch fecundity. The formation of the new batch at the end of spawning showed that annual fecundity of *L. dieuzeidei* is indeterminate. Absolute batch fecundity was estimated to be from 558 to 10 230 oocytes per female. Fecundity was positively correlated with total length \( F = 1.181 \times TL^{3.35} \), \( r^2 = 0.750 \), Fig. 9a), total weight \( F = 252.5 \times TW^{1.04} \), \( r^2 = 0.748 \), Fig. 9b) and gonad weight \( F = 3643.7 \times GW^{0.90} \), \( r^2 = 0.904 \), Fig. 9c). The best correlation was established for fecundity-gonad weight relationship \( r^2 = 0.904 \). Comparing fecundity with age, Tukey test (ANOVA) showed that there is significant similarity \( P < 0.0001 \) between age 3 and 4 (Fig. 10).

Histological analysis of gonads in mature males showed that spermatocysts of *L. dieuzeidei* were containing all stages of spermatogenesis including spermatids and spermatozoa throughout the whole year. Presence of the spermatozoa in the lumen of the lobules and in the sperm ducts during September (Fig. 11a) and April (Fig. 11b) indicated spawning capable phase following the peaks in the females spawning season.
DISCUSSION

This study represents the first detailed description of the reproductive characteristics of *L. dieuzeidei*. This species is considered as by-catch in trawl nets in the Adriatic Sea and therefore fishing effort is one of the key factors influencing the composition of its population.

The selectivity of fishing gear used in this study may be the reason why individuals smaller than 7 cm were not caught. The total length of analysed individuals ranged from 7.46 to 14.69 cm which is similar to those obtained in other areas (OLIM & BORGES, 2006; VALLISNERI, et al. 2011; TORRES et al., 2012). Another reason is that the juveniles of this species, as well as of other species belonging to the family Triglidae, only live in shallow areas and their habitat change is related with growth and reproduction (PAPACONSTANTINOU, 1983; COLLOCA et al., 1994, 1997). The maximum total length of 20.4 cm was reported in the northeastern Mediterranean (BAŞUSTA et al., 2013a).

The dominance of females recorded in this study was already observed for the majority of species within the Triglidae family (İLHAN & TOĞULGA, 2007; BOUDAYA et al., 2008; BEN JRAD et al., 2010; MARROTT, et al., 2010; VALLISNERI et al., 2011; DOBROSLAVIĆ et al., 2015). Fishing time and used fishing gear can enhance further fluctuation of male to female ratio in the population (RICKER, 1973). Positive allometry was obtained in this study as well as in previous studies of *L. dieuzeidei*. Equation parameters for females and males from different studies are given in Table 1.

Females had higher values of condition factor due to higher gonad weight. Higher values for females (1.39) than males (1.21) were also recorded in the study of OLIM & BORGES (2006). BAŞUSTA et al. (2013a) obtained smaller values of condition factor for females (0.80) than males (0.81) which they related to gonadal development. Results from this study showed that values followed the reproductive cycle with lower values recorded during the spawning period. High and low values were recorded both at higher and smaller total lengths indicating spawning and maturation.
Earlier sexual maturation of males is common in the majority of species including the family Triglidae. (PAULY, 1978, 1994; PAPACONSTATINOU, 1984). This was recorded for tub gurnard Che
donichthys lucerna (İLHAN & TOĞULGA, 2007; BOUDAYA et al., 2008; VALLISNERI et al., 2011; EL-SERAFY et al., 2015), red gurnard Chelidonichthys cuculus (VALLISNERI et al., 2014) and longfin gurnard Chelidonichthys obscurus (MUÑOZ et al., 2001; MUÑOZ et al., 2003). In this study female of L. dieuzeidei reach sexual maturity at a smaller total length (8.99 cm) compared to males (10.01 cm). Earlier maturation of females is usually observed in populations with a smaller number of males than females (BAYLIS, 1981). Maturation of females at smaller total lengths than males is also recorded for streaked gurnard, Chelidonichthys lastoviza (BEN JARD et al., 2010). Length at first maturity is a good indicator of species vulnerability to fishing impact (ANDERSON et al., 2008) as over-exploitation of immature individuals could have a negative impact on the population resilience and recruitment (MYERS et al., 1997).

L. dieuzeidei in the south Adriatic shows a prolonged spawning period (November to July), which is common among gurnards (LEWIS & YERGER, 1976; MCBRIDE & ABLE, 1994). This observation is in accordance with the results of a previous study on L. cavillone in the Adriatic Sea which reported maximum gonadal activity between January and August (DOBROSLAVIĆ et al., 2015). According to available literature spawning period in other Triglidae is shorter (MUÑOZ et al., 2001; MUÑOZ et al., 2003; İLHAN & TOĞULGA, 2007; BOUDAYA et al., 2008; VALLISNERI et al., 2014; EL-SERAFY et al., 2015) than those recorded for species of gender Lepidotrigla in the Adriatic Sea. Also, the male gonads of L. dieuzeidei were found significantly smaller than female gonads; the same characteristic was observed in previous studies of L. cavillone (COLLOCA et al., 1997; İLKAYAZ et al., 2010; DOBROSLAVIĆ et al., 2015).

The reproductive cycle was assessed using standard qualitative and quantitative histological techniques as the primary method for staging gonadal development. Based on monthly gonad analysis, this study showed that L. dieuzeidei has multiple synchronous development of ovaries with multiple spawning events. This was also recorded for longfin gurnard C. obscura (MUÑOZ et al., 2001) and large-scaled gurnard L. cavillone (DOBROSLAVIĆ et al., 2015). The ovaries were always represented with a group of primary oocytes and a diverse population of vitellogenic oocytes, which are each specially recruited during the final stages of maturation in uniform or uneven phases. Also, postovulatory tissue in the gonads with vitellogenic oocytes supports the previous statement that the species spawns several times within the spawning period. Multiple spawning is a strategy to increase larvae survival (LAMBERT & WARE, 1984) and it also occurs in species with high fecundity and a physical limitation (small size), since hydration significantly increases the diameter of the eggs and expands the abdominal cavity (BEGENAL, 1973; FORDHAM & TRIPPEL, 1999). Atresia is influenced by several external factors, temperature changes, lack of food and stress being the most important ones (GURAYA & SAUER, 1986). Since this species lives at depths were the external factor are stable we

### Table 1. Length-weight relationship equation constant of Lepidotrigla dieuzeidei from different locations

| Authors                  | Location                        | Sex | a     | b     | R²   |
|--------------------------|---------------------------------|-----|-------|-------|------|
| Olim and Borges (2006)   | south coast of Portugal         | F   | 0.011 | 3.04  | 0.87 |
|                          |                                 | M   | 0.009 | 3.11  | 0.96 |
| Vallisneri et al. (2010) | Adriatic Sea                    | F   | 0.005 | 3.27  | 0.95 |
|                          |                                 | M   | 0.006 | 3.31  | 0.96 |
| Başusta et al. (2013)    | northeast Mediterranean Sea      | F   | 0.003 | 3.41  | 0.84 |
|                          |                                 | M   | 0.004 | 3.28  | 0.81 |
| This study               | Adriatic Sea                    | F   | 0.082 | 3.11  | 0.90 |
|                          |                                 | M   | 0.009 | 3.04  | 0.93 |
assume that a high number of atretic oocytes is the natural process of regulating final fecundity. Atresia has been described as a regulation process to maintain ovary homeostasis and it represents the ratio between the number and size of oocytes and the available energy sources (LUCKENBACH et al., 2008).

A quantitative method of measuring the size of oocytes (expressed as oocyte diameter) was also employed in this study and it followed the pattern observed using qualitative histological methods. The maximum oocyte diameter recorded for *L. dieuzeidei* was 946.05 µm, which coincides with the maximum size report for *L. cavillone* at 974.85 µm (DOBROSLAVIĆ et al., 2015). The quantitative analysis of female gonads showed a continuous representation of all oocyte sizes throughout the year with no visible gaps between the first and the second stage of development, meaning that previtellogenic and vitellogenic oocytes were always present in the gonads.

The continuous first and second developmental stage of oocytes indicate the indeterminate type of fecundity (for this species, with a prolonged spawning period and spawning occurring several times in a year HUNTER & MACEWICZ, 1985b; WEST, 1990). Species that have such indefinite fecundity continuously produce new oocytes for the entire period of spawning, and as such, it greatly depends on environmental factors (e.g. temperature and food availability) and the overall length and weight of the body (HUNTER et al., 1985).

*L. dieuzeidei* has a lobular type of testes, recorded also for other triglids (MUÑOZ et al., 2001; DOBROSLAVIĆ et al., 2015). The analysis from this study showed that male gonads were at the spawning stage almost all year round, with spermatogenetic cells in different developmental stages. This indicates a long spawning period of several months to one year (SCHULZ et al., 2010).

By-catch species have an important role in the energy flow and uncontrolled harvesting of these species can have negative consequences especially in the oligotrophic seas such as Adriatic. To be able to answer the question is this type of reproduction characteristic for *L. dieuzeidei* or it is the answer to a high fishing effort future monitoring of this species is required.

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Reproduktivna strategija kokotića, *Lepidotrigla dieuzeidei* Blanc and Hureau, 1973 iz jugoistočnog dijela Jadranskog mora

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SAŽETAK

Ovo istraživanje donosi prve podatke o reproduktivnoj biologiji kokotića, *Lepidotrigla dieuzeidei*. Ukupno je prikupljeno 1027 jedinki, uzorkovano je mjesečno od rujna 2011. do kolovoza 2012. Ukupna duljina jedinki bila je u rasponu od 7,46 cm do 14,62 cm s omjerom spolova ženke:mužjaci 1:0,59. Pozitivan alometrijski rast zabilježen je i kod ženaka \( b = 3,07 \) i kod mužjaka \( b = 3,08 \). Promjene indeksa kondicije bile su slične kod oba spola sa srednjim vrijednostima od 1,07 za ženke i 1,01 za mužjake. Temeljem mjesečnih vrijednosti gonadosomatskog indeksa utvrđeno je da kokotić ima dugo razdoblje mriješćenja, od studenog do srpnja. Histološka analiza pokazala je multipli sinkroni razvoj jajnika s više razdoblja mriješćenja. U jajnicima su uvijek bile zastupljene skupine primarnih oocita i raznovrsni stadiji vitelogenih oocita, koji su regrutirani u posljednjim stadijima sazrijevanja u jednoličnim ili neravnomjernim fazama. Fekunditet je procijenjen gravimetrijski primjenom metode hidratiziranih oocita i iznosi oko 558 do 10230 oocita po ženki. Kokotić je važna vrsta u prilovu i podaci iz ovog istraživanja predstavljaju značajan doprinos upravljanja stockom.

**Ključne riječi:** oogeneza; spermatogeneza; sazrijevanje gonada; plodnost; promjer oocita; višestruko mriješćenje ribe