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## Effect of light intensity on the survival of European spiny lobster (*Palinurus elephas*) larvae reared in aquaculture system

Jean-José Filippi<sup>a,\*</sup>, Rémi Millot<sup>a,\*</sup>, Jérémy Bracconi<sup>a</sup>, Viviana Ligorini<sup>a</sup>, Nicolas Gattacceca<sup>a</sup>, Ambre Crescioni<sup>a</sup>, Jean-Baptiste Ronchi-Perfetti<sup>a</sup>, Mikaël Demolliens<sup>a</sup>, Sally Pugliese<sup>a</sup>, Alban Delmas<sup>a</sup>, Alizée Boussard<sup>a</sup>, Alexandre Vela<sup>a</sup>, Romain Bastien<sup>a</sup>, Vanina Pasqualini<sup>a,b</sup>, Antoine Aiello<sup>a,b</sup>

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### ABSTRACT

Light plays a crucial role in the growth, development, and survival of aquatic organisms. To evaluate the survival of European spiny lobster (*Palinurus elephas*) larvae in relation to different light intensities (26, 359, and 910 lux, equivalent to 0.35, 4.74, and 12.29  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively), this study monitored the survival rates of reared spiny lobster from the early instar phyllosoma stages to their metamorphosis into the puerulus stage (111 days after hatching) at the three light intensities. The newly hatched larvae were cultured in 65-L Kreisel tanks with 200 individuals each (three replicates per treatment, *i.e.*, light intensity). Phyllosoma stages I to III larvae showed no significant differences in survival rates under different light intensities. However, light intensity did have a significant impact on the survival rates of phyllosoma stages IV and V as well as the metamorphosis of phyllosoma VI into puerulus. The highest survival rates were observed under high and medium light intensity conditions. At the end of the experiment, the highest mean survival rate for stage VI, the metamorphosis into the puerulus stage, was  $42.0 \pm 2.52\%$  under high light intensity. These survival rates for reared pueruli were 84 times higher than those achieved in previous studies. The present study results show promise for the successful implementation of commercial scale rearing protocols for European spiny lobster and have implications for the design of crustacean larvae culture systems.

### 1. Introduction

The European spiny lobster *Palinurus elephas* (Fabricius, 1787) is a prominent crustacean with a wide distribution. It can be found along the coasts of the northeast Atlantic, from southern Norway to Mauritania, including the Canary Islands and the Azores, as well as the Mediterranean Sea; however, it is absent from the extreme eastern and south-eastern basins of this sea (Goñi and Latrouite, 2005). European spiny lobster primarily inhabits rocky seabeds and is commonly found in association with diverse coralligenous communities at depths ranging from 0 to 200 m (Campillo and Amadei, 1978; Yeap et al., 2022). The European spiny lobster is of great environmental, economic, and societal importance in the Mediterranean Sea. *P. elephas* is a valuable fishery resource that is subject to intense fishing pressure. Its selling price

fluctuates between 50 and 100 €/kg (approximately \$55–109 US) depending on the time of year and the type of sale (*i.e.*, via fishmongers, restaurants, or individuals) (Marengo et al., 2020). According to the FAO's capture production statistics, the capture of *P. elephas* along European coasts has experienced a sharp decline, dropping from 1100 tonnes in 1969–434 tonnes in 2017 (Marengo et al., 2020). In France, landings along the Atlantic coast decreased from 1000 tonnes/y in the 1950s to 25 tonnes in 2010 (Goñi, 2014). This decline in lobster abundance has created a feedback loop, further exacerbating the species' decline. The relative scarcity of the species has led to an increase in fishing effort, which involves the use of longer nets, more boats, and fishing in deeper fishing grounds. This has had significant negative impacts on biodiversity, including increased bycatch, greater disturbance of the seabed, and higher greenhouse gas emissions per spiny

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lobster caught (Parker et al., 2018). Consequently, the European spiny lobster is listed as a “vulnerable” species on the International Union for Conservation of Nature (IUCN) red list (Goñi, 2014).

A more sustainable and long-term strategy for meeting growing market demands is farming spiny lobsters (Williams, 2007). Furthermore, the production of farmed juveniles could help restore wild populations through stock enhancement programs similar to those implemented for the Chesapeake Bay blue crab (*Callinectes sapidus*) (Zohar et al., 2008), the swimming crab (*Portunus trituberculatus*) (Ariyama, 2001), and the common European lobster (*Homarus gammaurus*) (Williams, 2007). The aquaculture of spiny lobsters represents an opportunity to enhance lobster fisheries—most global stocks are currently fully exploited—while promoting a sustainable exploitation of this resource. However, spiny lobster aquaculture is hindered by the low survival rate of phyllosoma larvae during hatchery rearing through to the metamorphosis stage (Kittaka and Booth, 1994). Complete development from egg to puerulus has been achieved for several palinurid species, with a development length ranging from 205 to 319 days for *Jasus* spp. and 306–341 days for other *Palinurus* spp. (Booth, 1996; Kittaka, 1988; Kittaka et al., 1988, 1997; Kittaka and Kimura, 1989; Matsuda and Yamakawa, 2000; 1989). There is no equivalent restocking programs or commercial aquaculture ventures for European spiny lobster. Very little research and published data are available regarding hatchery procedures for this species (Kittaka and Ikegami, 1988; Kittaka et al., 2001); much of the available information is in the form of grey literature, such as the University of Vigo (Universidad de Vigo, 2015), the french startup LAGOSTA (LAGOSTA research, 2024) and of Dr David Fletcher, aquaculture consultant for RAS Aquaculture Research Ltd (RASAR) (The Fish site, 2022). The limited availability of relevant information is not due to a lack of demand, but rather a lack of biological knowledge, particularly regarding the complex larval development, and underdeveloped farming practices for the species. Consequently, in the absence of specific information, researchers often draw upon parallels from studies conducted on similar species, such as the rock lobster (*Jasus edwardsii*) (Jeffs and James, 2001).

Although extensive research has been conducted on the environmental requirements of larval cultures (Bermudes and Ritar, 2008 (Spiny rock lobster, *Jasus edwardsii*); Matsuda and Yamakawa, 2000 (Longlegged spiny lobster, *Palinurus longipes*); Moss et al., 1999 (Spiny rock lobster, *Jasus edwardsii*); Tong et al., 1997 (Spiny rock lobster, *Jasus edwardsii*)), the optimal environmental conditions for elevated growth and survival of spiny lobster species remain unknown. Light is a fundamental variable in aquaculture, as it regulates the survival, growth, and development of crustaceans (Dou et al., 2021). Light is one of the primary orienting stimuli that control the directional response of animals in the vertical plane (along with gravity) (Fraenkel and Gunn, 1961). However, light varies greatly in the marine environment, and its characteristics and intensity, along with temperature and salinity, can affect a species' type and level of response (Sulkin, 1984). For example, bright light shortens the intermolt duration of Australian giant crab (*Pseudocarcinus gigas*) larvae (Gardner and Maguire, 1998), increases the molting frequency of banana prawn (*Penaeus merguensis*) broodstock (Hoang et al., 2002, 2003), and enhances the survival rates of some stages of swimming crab (*Portunus trituberculatus*; Dou et al., 2021). In contrast, high light intensity negatively affects the metabolism of the spiny rock lobster (*Jasus edwardsii*; Bermudes et al., 2008) and the growth performance and energy allocation in Chinese white shrimp (*Fenneropenaeus chinensis*; Wang et al., 2004).

To the author's, no advancements in optimizing hatchery operations or increasing survival rates have been published for more than twenty years since Kittaka et al. (2001). Survival rates in culturing phyllosoma remain poorly constrained (Kittaka and Ikegami, 1988; Kittaka, 1994a, b; Kittaka, 1997a, b). In Corsica (France), the focus has been on developing aquacultural rearing techniques for European spiny lobster. The goal is to increase the number of produced juveniles to eventually supply juveniles in sufficient quantities for ecological restoration experiments

in the Mediterranean basin. The objectives of this study were to (i) determine through experimental assays how light intensity affects the success of *P. elephas* larvae rearing and (ii) review the available information on reared *P. elephas*.

## 2. Materials and methods

### 2.1. Broodstock collection and incubation

European spiny lobster berried females' captures were approved in compliance with Order No. R20–2022–09–05–00003 dated 05/09/2022 from the Direction of the Sea and Corsican Coast, authorizing personnel from the UAR 3514 Stella Mare marine research center of the University of Corsica-CNRS, Biguglia, France, to collect biological samples for scientific purposes (berried females captures and landings are otherwise prohibited in France). Since 2006, the European Council regulation (EC, No 1967/2006) on professional fishing prohibits the capture and landing of *P. elephas* berried females and sets the minimum catch size at 90 mm cephalothorax length (CL) for the European Mediterranean Sea. The collected broodstock comprised twenty wild ovigerous females ( $707.6 \pm 188.4$  g, mean  $\pm$  SD) captured in 2022 during the spawning period (i.e., from August to September) by four Corsican artisanal vessels. Seven females ( $544.6 \pm 159.7$  g, mean  $\pm$  SD) were captured by two commercial fishermen on the west coast of Corsica and 13 ( $795.3 \pm 140.1$  g, mean  $\pm$  SD) were captured by two other commercial fishermen on the Corsican east coast (Fig. 1). These broodstock were captured using spiny lobster nets (trammel or gillnet, generally of a mesh size between 4 and 6) for a minimum of 48 hr at depths up to 200 m. Particular attention was paid to avoiding shocks or stress to the females after their capture. Within 3 hr of capture, the specimens were transported alive to the Stella Mare marine research center of the University of Corsica-CNRS (Biguglia, France) inside an icebox to avoid thermal shock. At the hatchery, berried females were examined for any wounds or missing anatomical parts and transferred to two 2.5 m<sup>3</sup> semi-square flat-bottom fiberglass tanks (10 broodstock per tank) with a recirculated aquaculture system (RAS) providing 0.1  $\mu$ m filtered seawater. Shelters made of PVC were provided for each female as a refuge. The berried spiny lobster females were fed *ad libitum* with a mixed diet of frozen small fish, mussels, prawns, and squid. During the egg-laying period (i.e., from early December 2022 to early January 2023), newly hatched larvae from a selected female were carefully collected. Dead or moribund larvae swimming near the bottom of the tanks were discarded to avoid bad batches or contamination at the onset of larval rearing.

### 2.2. Experimental design

The newly hatched larvae (phyllosoma stage I, i.e., "begin") ( $n = 1800$ ) were randomly assigned to a 65 L semicircular Kreisel tank, and 200 larvae were placed into each tank. Three different light intensities were selected: low (26 lux, i.e.,  $0.35 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), medium (359 lux, i.e.,  $4.74 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ), and high (910 lux, i.e.,  $12.29 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ); each intensity had three replicates. Light intensities were regularly monitored above the water surface using a light meter (PCE Holding, Hamburg, Germany). The photoperiod was 12 L:12D, and the light sources were Luxina T8 LED 21-Watt lamps (Luxna Lighting, Chaponost, France) placed above the water surface. All replicates were placed in separate rooms to avoid light contamination among treatments. The experiments were performed in a dark room to prevent the entry of natural light.

### 2.3. Larval rearing

The design of the Kreisel tank has water entering the tank via a bottom central inlet and exiting via a top central drain. This creates a circular current that keeps the larvae in the water column, apart from one another while also prevents the sinking of the larvae. To maintain

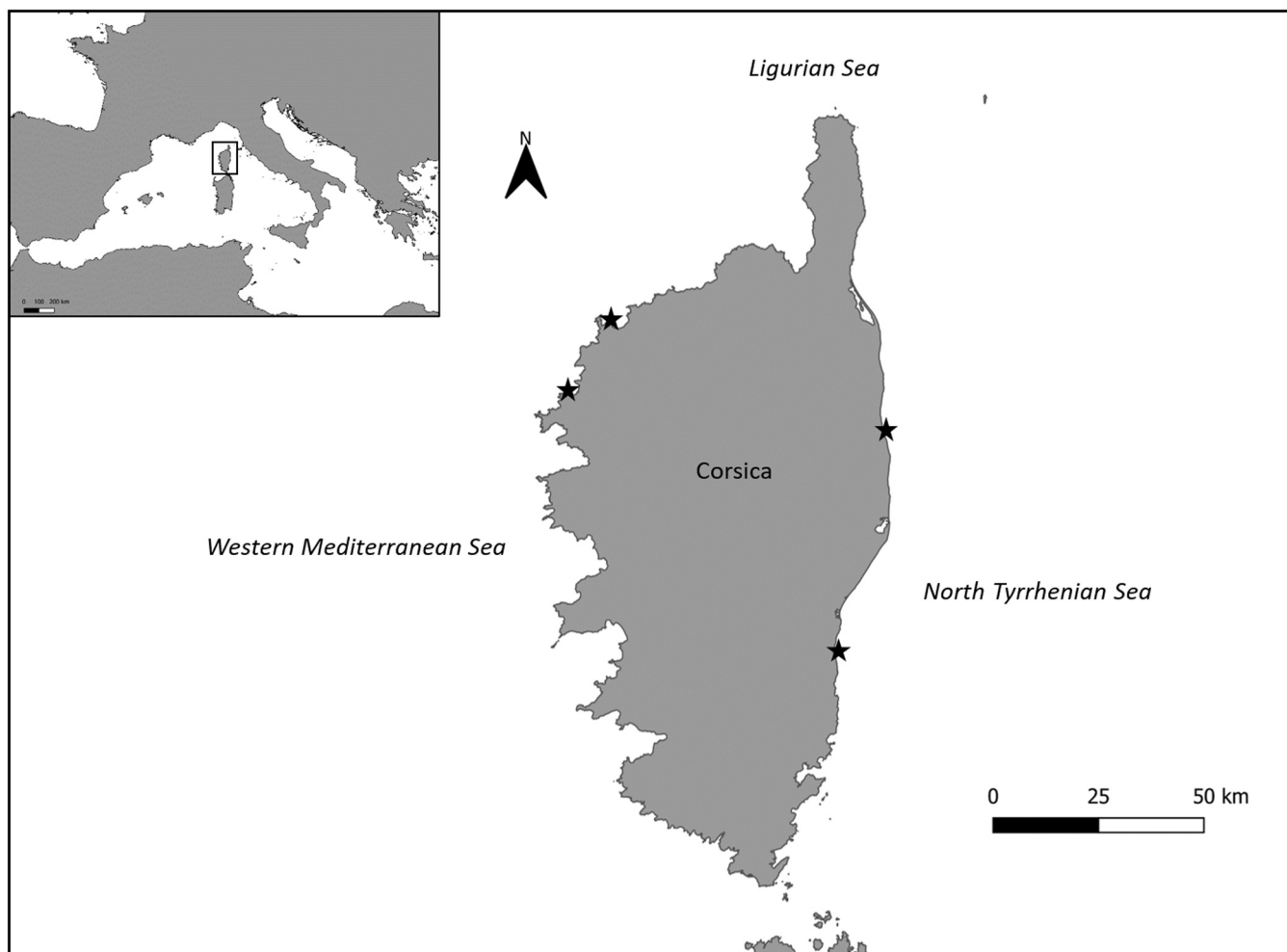


Fig. 1. Capture sites (★) of *Palinurus elephas* ovigerous females, August to September 2022.

this flow, water renewal was set at 108 L·h<sup>-1</sup> in these tanks, at the beginning of the larval rearing. In each tank, a 100 µm mesh screen was added to the water outlet system to avoid loss of phyllosomas or food. Water temperature (15.26 ± 0.35 °C) and water quality (pH: 7.84 ± 1.07, nitrite < 0.001 mg·L<sup>-1</sup>, nitrate < 0.001 mg·L<sup>-1</sup> and ammonia < 0.001 mg·L<sup>-1</sup> concentrations, dissolved oxygen: 99.70 ± 3.40 %, total gas: 100.55 ± 0.50 %, and residual gas pressure: 100.77 ± 0.89 %) were monitored continuously throughout the experiment using Oxyguard Handy Polaris 2 and TGP probes (Oxyguard International, Farum, Denmark). Dead and moribund larvae were counted and removed daily. Larvae were fed with newly hatched common dentex fish larvae (*Dentex dentex*) obtained from the Stella Mare marine research center of the University of Corsica-CNRS (Biguglia, France). Moribund fish larvae were automatically discarded from the process to avoid contamination of the phyllosomas.

#### 2.4. Experimental procedure and sample collection

In each tank, once a phyllosoma molted to the next stage, it was removed and transferred to a running identical empty tank to avoid cannibalism. Dead larvae were counted once all larvae had molted. Thus, transfers occurred 20, 28, 42, 60, 85, and 111 days after hatching (DAH) and corresponded to the larvae molting to the following stage (0 DAH: phyllosoma stage I, *i.e.*, "Begin"; 20 DAH: "Phy I," *i.e.*, phyllosoma I molt to phyllosoma II; 28 DAH: "Phy II," *i.e.*, phyllosoma II molt to phyllosoma III; 42 DAH: "Phy III," *i.e.*, phyllosoma III molt to phyllosoma IV; 60 DAH: "Phy IV," *i.e.*, phyllosoma IV molt to phyllosoma V; 85 DAH:

"Phy V," *i.e.*, phyllosoma V molt to phyllosoma VI; 111 DAH: "Phy VI," *i.e.*, phyllosoma VI metamorphosis to puerulus). During the experiment, the larvae underwent six phyllosoma stages and metamorphosis to the puerulus stage. The experiment ended when all larvae had either died or had reached the puerulus stage. Following the experiment, observations were made after the puerulus stage over a few weeks to quantify and assess the survival of the puerulus passing through the various juvenile stages.

#### 2.5. Survivability calculations

The cumulative survival rate and staged survival/mortality rate were calculated as

$$\text{Cumulative survival rate(\%)} = O_s \times O_1^{-1} \times 100, \tag{1}$$

$$\text{Staged survival rate(\%)} = O_s \times O_1^{-1} \times 100, \tag{2}$$

$$\text{Staged mortality rate(\%)} = [(O_s \times O_1^{-1}) - 1] \times 100, \tag{3}$$

where  $O_i$  is the number of phyllosoma stage I larvae at the beginning of the experiment (200, 0 DAH),  $O_s$  is the number of larvae that successfully molted to each stage, and  $O_1$  is the number of larvae in the previous stage of  $O_s$ .

#### 2.6. Statistical analysis

All statistical analyses were performed using R software v.4.2.0 (R

Core Team, 2022). All data are presented as the mean  $\pm$  standard error (SE) (three replicates per treatment). The normality and homogeneity of variances were analyzed by examining residual values using the Shapiro-Wilk's and Levene's tests, respectively. The non-normal and heterogeneous data were transformed with arcsine or log transformations to stabilize variation (Snedecor and Cochran, 1989). Differences among light intensity treatments were tested by one-way ANOVA, with the separation of treatment means by Tukey's honestly significant difference (HSD) tests. To compare survival rates over time for each light intensity, treatments were analyzed using the Kaplan–Meier survival analysis with a Weibull distribution of errors using the R package 'survival' (Therneau and Grambsch, 2000; Therneau, 2015). The Mantel–Cox test results were represented by Kaplan–Meier curves produced using the package 'survminer' (Kassambara et al., 2021). The Mantel–Cox test or log-rank test is a nonparametric and hypothesis-based test for comparing the distribution of survival curves of at least two samples. The resulting *P*-values were adjusted using Tukey's method for multiple pairwise comparisons of survival curves by treatment. The time to 50 % mortality of *P. elephas* larvae (LT<sub>50</sub>, defined as the time for 50 %

of the individuals to die) for each treatment combination was fitted on days 20–111 to a four-parameter log-logistic regression model ( $n = 3$ ) using the *drm()* function in the 'drc' package (version 1.0–1). Null hypotheses were rejected when  $P < 0.05$ . An abnormal peak of mortality was observed for replicate 3 at 85 DAH for the 359 lux treatment; this sample was removed from the data set to avoid statistical bias. The real *p*-values for all the tests are indicated in [supplementary material \(Table S1 et S6\)](#).

### 3. Results

#### 3.1. Effects of light intensity on inter-stage and cumulative survival

The cumulative survival rates of *P. elephas* under different light intensities for Phy I to Phy III presented no significant differences among the treatments (ANOVA,  $P > 0.05$ ; i.e., Phy I:  $F = 2.286$ ;  $df = 2$ ;  $P = 0.197$ , Phy II:  $F = 2.992$ ;  $df = 2$ ;  $P = 0.140$ , Phy III:  $F = 4.907$ ;  $df = 2$ ;  $P = 0.066$ , Table S1) (Fig. 2a, Table 1). From Phy IV to Phy VI, significant differences among treatments were observed (ANOVA,  $P < 0.05$ ; i.e.,

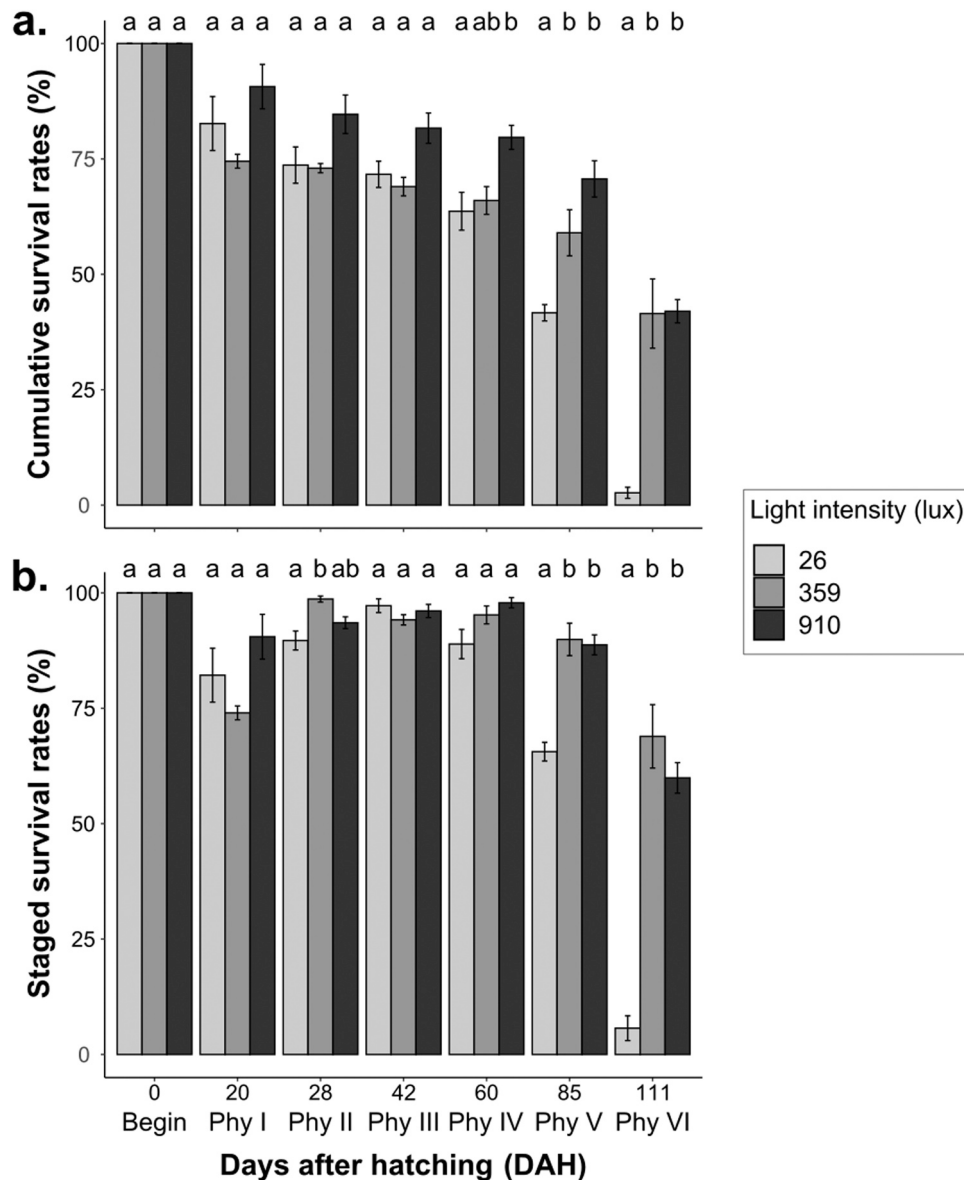


Fig. 2. Mean ( $\pm$  SE) (a) cumulative (b) and staged survival rate of *Palinurus elephas* larvae reared under different light intensities. Bars with the different letters on particular days after hatching (DAH) indicate statistically significant differences ( $P < 0.05$ ).

**Table 1**

Cumulative survival rate of *Palinurus elephas* larvae (% mean ± SE) by development stage (or reared time, days after hatching; DAH) under different light intensities; low: 26 lux, medium: 359 lux, high: 910 lux.

Time (DAH)	Stage	Light intensity		
		Low	Medium	High
0	Begin	100 ± 0.00	100 ± 0.00	100 ± 0.00
20	Phy I	82.67 ± 5.84	74.50 ± 1.50	90.67 ± 4.81
28	Phy II	73.67 ± 3.93	73.00 ± 1.00	84.67 ± 4.18
42	Phy III	71.67 ± 2.85	69.00 ± 2.00	81.67 ± 3.28
60	Phy IV	63.67 ± 4.10	66.00 ± 3.00	79.67 ± 2.60
85	Phy V	41.67 ± 1.76	59.00 ± 5.00	70.67 ± 3.93
111	Phy VI	2.67 ± 1.20	41.50 ± 7.50	42.00 ± 2.52

Phy IV:  $F = 6.781$ ;  $df = 2$ ;  $P = 0.038$ ; Phy V:  $F = 19.73$ ;  $df = 2$ ;  $P = 0.004$  and Phy VI:  $F = 45.10$ ;  $df = 2$ ;  $P = 0.001$ , Table S1). The cumulative survival rates for the 910 lux and 359 lux treatments were significantly higher than for 26 lux (Tukey's HSD,  $P < 0.05$ , Table S1). The exception was Phy IV, which did not differ between the 26 and 359 lux treatments (Tukey's HSD,  $P = 0.895$ , Table S1).

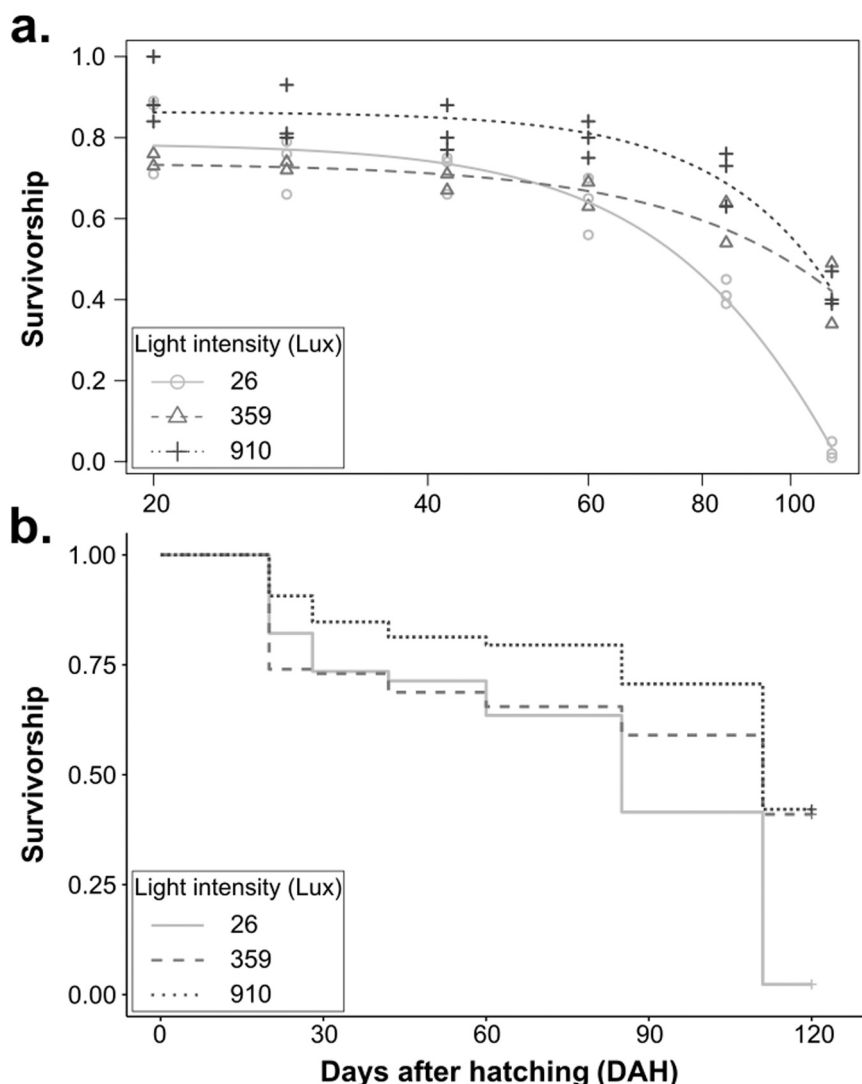
The survival rate of *P. elephas* at different developmental stages (or days after hatching (DAH)) and light intensities presented no significant differences for Phy I (20 DAH), Phy III (42 DAH) and Phy IV (60 DAH) among the light intensities (ANOVA,  $P > 0.05$ ; i.e., Phy I:  $F = 2.369$ ;  $df =$

2;  $P = 0.189$ , Phy III:  $F = 6.743$ ;  $df = 2$ ;  $P = 0.430$ , Phy IV:  $F = 4.158$ ;  $df = 2$ ;  $P = 0.086$ , Table S2) (Fig. 2b). Nevertheless, significantly higher survival rates were observed at 359 lux and 910 lux than at 26 lux for Phy II, Phy V, and Phy VI (ANOVA,  $P < 0.05$ ; i.e., Phy II:  $F = 6.743$ ;  $df = 2$ ;  $P = 0.038$ , Phy V:  $F = 33.81$ ;  $df = 2$ ;  $P = 0.001$ , Phy VI:  $F = 78.31$ ;  $df = 2$ ;  $P = 0.20e^{-3}$ ; Tukey's HSD,  $P < 0.05$ , Table S2) (Fig. 2b).

**3.2. Effects of light intensity on survival timing**

The Kaplan–Meier survival curves (Fig. 3b) illustrate that 50 % of larval *P. elephas* mortality occurred between 85 and 111 DAH for all light intensity treatments. The survival curves for all treatments differed significantly (log-rank:  $\chi^2 = 238$ ;  $df = 2$ ,  $n = 1600$ ;  $P < 2e^{-16}$ ). The 910 lux light intensity treatment produced low mortality relative to the 26 lux (Tukey's HSD,  $P < 2e^{-16}$ ) and 359 lux light intensity treatments (Tukey's HSD,  $P < 0.043$ ). The 359 lux treatment produced lower larvae mortality than the 26 lux treatment (Tukey's HSD,  $P < 2e^{-16}$ ) (Table S3).

Mortality estimates using log-logistic regression (Fig. 3a) fell within the ranges observed using the Kaplan–Meier model, with the time to 50% mortality (LT<sub>50</sub>) ranging from 76.1 to 105 DAH (Table 2). The estimated time to 50 % mortality varied significantly among light intensity treatments. LT<sub>50</sub> of *P. elephas* larvae was faster for 26 lux than for the other light intensity treatments.



**Fig. 3.** *Palinurus elephas* larvae survival per light intensity treatment, calculated using (a) Kaplan–Meier survival analysis and (b) log-logistic regression.

**Table 2**

Time to 50 % mortality (LT<sub>50</sub>) with a 95 % confidence interval for *Palinurus elephas* larvae under different light intensities. Different letters represent significant differences ( $P < 0.05$ ).

Light intensity (lux)	LT <sub>50</sub> (95 % CI) (day)
26: low intensity	76.1 (69.5–82.6) <sup>a</sup>
359: medium intensity	98.4 (86.1–110.7) <sup>b</sup>
910: high intensity	105.0 (96.9–111) <sup>b</sup>

### 3.3. Pattern of staged mortality rate

Mortality at each development stage (Fig. 4a, b, c) varied significantly for all treatments (ANOVA,  $P < 0.05$ ; i.e., 26 lux:  $F = 23.09$ ;  $df = 6$ ;  $P = 1.67e^{-6}$ , 359 lux:  $F = 45.07$ ;  $df = 6$ ;  $P = 3.04e^{-5}$ , 910 lux:  $F = 15.23$ ;  $df = 6$ ;  $P = 2.06e^{-5}$ ). For 26 lux (Tukey's HSD,  $P = 0.008$ ) and 359 lux (Tukey's HSD,  $P = 5.22e^{-5}$ ) treatments, a significant peak of mortality occurred during the first molt (Phy I; i.e., molt from phyllosoma I to phyllosoma II) (Table S4, S5). A similar pattern for the 910 lux treatment was observed, although it was not significant (Fig. 4c, Table S6). After the mortality peak, mortality decreased (Fig. 4a, b, c). The magnitude of this decrease varied among treatments, the highest variation in mortality being observed for the 359 lux treatment, where the staged mortality rate decreased from 25% (Phy I) to slightly above zero (Phy II) (Tukey's HSD,  $P = 6.76e^{-5}$ ). From Phy II to Phy IV, mortality tended to stagnate for all treatments. Finally, from Phy V to Phy VI, mortality tended to increase again, peaking at Phy VI with a significant difference for all treatments from Phy V to Phy VI (i.e., molt from phyllosoma VI to puerulus) (Tukey's HSD,  $P < 0.05$ , Table S4, S5, S6). For all treatments, mortality followed a U-shaped pattern (Fig. 4a, b, c).

## 4. Discussion

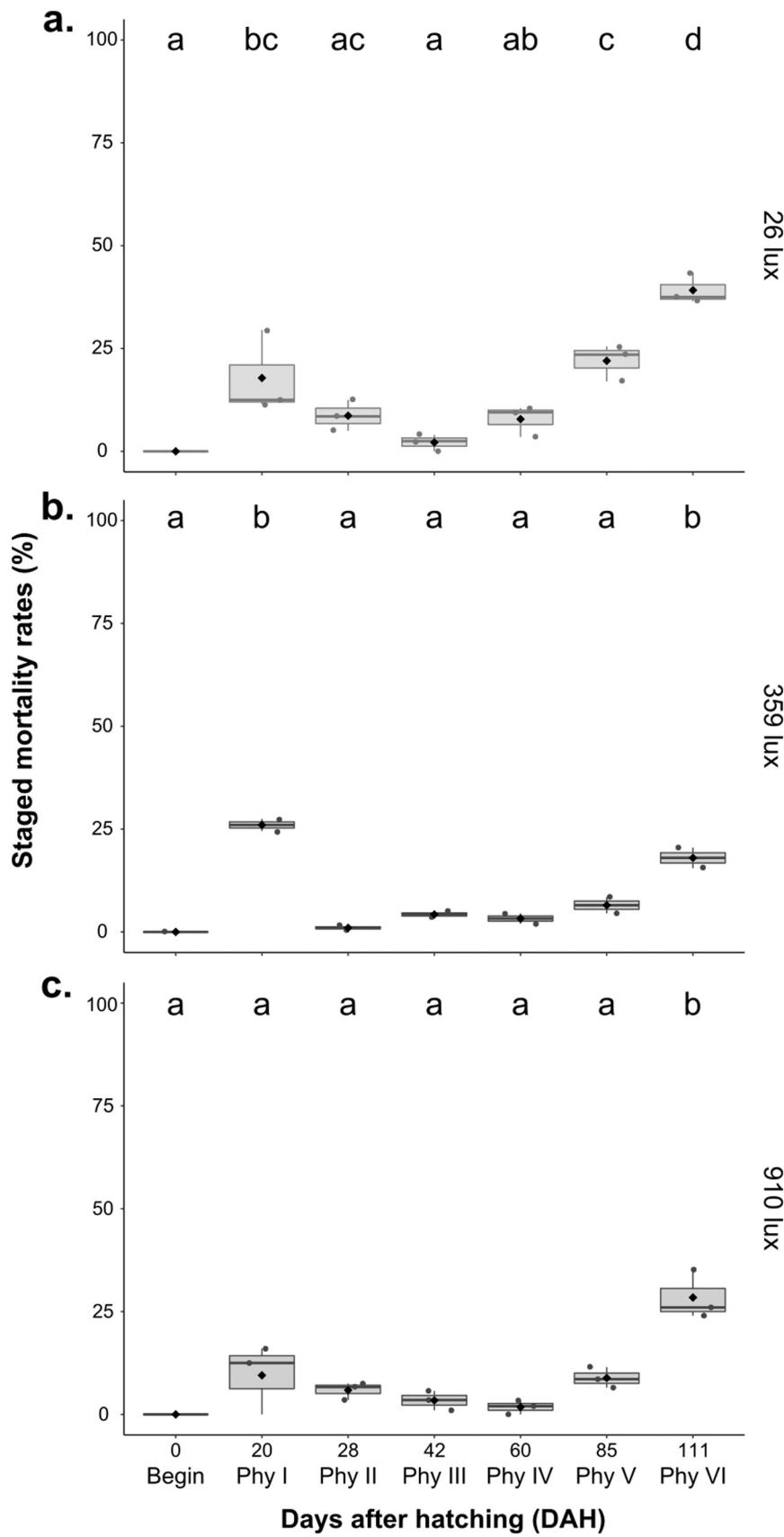
This study investigated the effect of light intensity on *P. elephas* larvae survival during hatchery rearing. Light is a key variable that influences the growth, development, and survival of aquatic organisms, including crustacean larvae (Bembe et al., 2018 (Blue crab, *Callinectes sapidus*); Chen et al., 2021 (Mud crab, *Scylla paramamosain*); Dou et al., 2021 (Swimming crab, *Portunus trituberculatus*); Guo et al., 2013 (Pacific white shrimp, *Litopenaeus vannamei*)). Nevertheless, there is limited information regarding the effects of light intensity on the survival, development, and growth of crustacean larvae, with few laboratory-based studies for aquacultural purposes relative to similar studies on larval fish.

In the present study, the effect of light intensity varied depending on the development stage of *P. elephas*. Early instars of phyllosoma were not significantly affected by light intensity, unlike the later stages and the metamorphosis of phyllosoma VI to puerulus. At these late stages, despite the higher mortality rate, high and intermediate (359 lux and 910 lux, i.e., 4.74 and 12.29  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) light intensities had higher survival rates than the low intensity treatment (26 lux, i.e., 0.35  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). Similar results were obtained for swimming crab (*Portunus trituberculatus*) (Dou et al., 2021), showing no effect of light intensity in the early stages (i.e., from Zoea I to Zoea III) but a high rate of larvae failing to complete their entire larval cycle at low light intensity and darkness during the later stages (i.e., Zoea IV and the metamorphosis to Zoea IV to megalopa).

Phyllosoma are less visual feeders and are more likely to capture prey through random encounters (Moss et al., 1999). Nevertheless, light stimulates swimming in the phyllosoma of the Palinuridae, Scyllaridae (Bermudes and Ritar, 2008; Mikami, 1995; Mikami and Greenwood, 1997; Ritz, 1972) and decapods in general. Less light would reduce larval activity and the rate at which larvae encounter prey (Andrés et al., 2010). Bermudes et al. (2008) and MacDiarmid (1985) demonstrated that newly hatched spiny rock lobster ( *Jasus edwardsii*) phyllosoma display strong, positively phototactic swimming toward the water

surface. Bermudes et al. (2008) reported that the swimming speed of phyllosoma is directly linked to light intensity, with a logarithmic increase in swimming speed as light intensity increases. Mikami (1995) also observed a similar pattern in the swimming speed of recently hatched flathead lobster (*Thenus orientalis*) larvae exposed to higher light intensities. The photokinesis mechanism can explain these observations, allowing larvae to adjust their locomotor activity in response to ambient lighting conditions (Bermudes et al., 2008). These adjustments may have energetic implications, potentially reducing growth and survival rates. Light intensity activates the swimming speed of phyllosomas for feeding intake, which affects the metabolism corresponding to metabolic feeding efficiency (feed intake: oxygen consumption ratio). A complex process occurs between the cost/benefit of the light intensity on larval development. Therefore, it is necessary to evaluate optimal light intensity because the response to light intensity appears to be species-dependent, making comparisons difficult among studies. According to Bermudes et al. (2008), the metabolic feeding efficiency is consistently higher in larvae exposed to low light intensities ( $7.7 \times 10^{12} \text{q}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$ ) than in phyllosoma in the dark or at high irradiance ( $3.9 \times 10^{14} \text{q}\cdot\text{s}^{-1}\cdot\text{cm}^{-2}$ ). These results agree with Moss et al. (1999), who found higher growth rates in spiny rock lobster (*Jasus edwardsii*) larvae fed under a low light intensity ( $0.001 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) than phyllosoma raised under high light intensity ( $10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The active swimming response could have contributed to a greater feed intake at the highest light intensity, possibly because of an imbalance between energy uptake and consumption (i.e., low metabolic feeding efficiency). In contrast, Dou et al. (2021) found that the survival rates of the swimming crab (*Portunus trituberculatus*) were highest for Zoea 4 and megalopa at 195.31 and 124.31  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , respectively, relative to survival at 0, 61.55, 93.57, 248.51, and 310.95  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Likewise, Tao et al. (2021) suggested that for giant freshwater prawn (*Macrobrachium rosenbergii*), a light intensity of 20,000 lux (i.e., 310.75  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) could improve survival rates and larval performance relative to 1000, 10,000, and 30,000 lux (i.e., 15.54, 155.38 and 466.13  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and natural light. Higher light intensities could compromise the survival rate. Possible explanations for this trend, could include (i) low light intensities do not increase the feeding rate or efficiency linearly and promote the feeding performance when it reaches a certain level; and (ii) high light intensities may be a stressor during the larval stages. Thus, the response to light intensity could be species-dependent, making comparisons difficult among studies.

In this study, the results showed no difference in the survival rate for the early instars of phyllosoma at all light intensity treatments. This absence of difference may stem from the metabolic feeding efficiency not having a negative impact regardless of the light intensity. Conversely, low light intensity may affect the metabolic feeding efficiency in the late stages and cause a low survival rate because the metamorphosis from phyllosoma to puerulus requires a significant intake. Most previous studies have tested only a few light intensities—generally two to four, e.g., Gardner and Maguire, (1998): 500 and 2 lux, i.e., 8.17  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and 0.03  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Australian giant crab, *Pseudosquilla carolinensis*); Hoang et al., (2002), (2003): 750 and 75 lux, i.e., 9.60  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and 0.99  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Banana prawn, *Penaeus merguensis*); Li et al., (2011): 134 and 14 lux, i.e., 1.73  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and 0.19  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Chinese mitten crab, *Eriocheir sinensis*). However, testing using a gradient of light intensities would be best for identifying the optimum intensity at which the feeding rate or efficiency improved feeding performance while not compromising the metabolic feeding efficiency. In the present study, higher light intensities produced the highest survival rates. However, optimal light intensity may not have been attained. Future research should add more light intensity levels considered low relative to intensities tested in existing studies. In this study, high intensity value of 910 lux (i.e., 12.29  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) is generally lower than previous studies' minimal low light intensity level (Dou et al., 2021; Tao et al., 2021). Moreover, the effect of light is multiple, not only the light intensity influences the individual



**Fig. 4.** Stage mortality rate of *Palinurus elephas* larvae under different development stages (or reared time, days after hatching (DAH)); (a) 26 lux, (b) 359 lux, and (c) 910 lux. Different letters indicate statistically significant differences ( $P < 0.05$ ).



performance parameters but also light quality/spectrum and duration (*i.e.*, photoperiod). Indeed, the photoperiod can influence survival, rate of development, and growth of larvae in several decapods (Bermudes and Ritar, 2008; Dou et al., 2021). The light quality/spectrum, specifically wavelength, can influence the animal behavior and growth performances, as it has been observed for New River crayfish (*Cambarus chasmodactylus*) and spiny stream crayfish (*Faxonius cristavarius*) which exhibit a stronger shielding behavior under red (620–750 nm) and blue (400–495 nm) light (Fischer, 2016). Also, Wei et al. (2021) found a better survival rate and metamorphosis rate for green light color for giant river prawn (*Macrobrachium rosenbergii*). However, in the present study the effect of photoperiod was not evaluated (12 L:12D) as well as the light quality/spectrum because only a white light was used (*i.e.*, a large spectrum lighting).

This study also aimed to optimize larval-rearing techniques. In the few existing studies of *P. elephas* larval development (Table 3), phyllosomas metamorphosed into pueruli at 132 DAH after an estimated nine instars (Kittaka and Ikegami, 1988), between 65 and 69 DAH after six instars (Kittaka et al., 2001), and 149 DAH (Kanemaru, 1992). All these studies obtained a low number of pueruli; *e.g.*, two and ten pueruli from 2000 first instars (*i.e.*, 0.1% and 0.5 %; Kittaka et al., 2001) and a single puerulus from 3000 first instars (*i.e.*, 0.03 %; Kittaka and Ikegami, 1988). Mercer et al. (1997) only managed to maintain phyllosoma to stage 4. These results of phyllosomas metamorphosing into pueruli at 111 DAH with a survival rate of 2.7–42 % are substantially higher when compared to previous studies showing six phyllosoma instars (similarly to Kittaka et al., 2001). The results obtained correspond to 84 times more pueruli than Kittaka et al. (2001) and 1400 times more pueruli than Kittaka and Ikegami (1988). For comparison, previously published survival rates are generally less than 1 %. Most of these studies assessed the effect of diet and concluded that phyllosomas of *P. elephas* had a higher survival rate in the early larval stage when they were fed with sailfin sandfish (*Arctoscopus japonicus*) larvae than when fed with mussel gonads and *Artemia* nauplii. *A. japonicus* has a high lipid content, particularly in highly unsaturated fatty acids (Kittaka and Booth, 2000). Indeed, Kittaka and Booth (2000) found 383,000 and 37,000 ng/mg dry weight of Eicosapentaenoic acid and docosahexaenoic acid content, respectively, while for *Artemia* nauplii the contents of these lipids were much lower, at 4700 and 76 ng/mg dry weight, respectively. One possible explanation of the high pueruli survival rate in this study could be the use of *Dentex dentex* fish larvae, which may have a better quality and caloric value as well as a lipid content equivalent to or even higher than that of *A. japonicus*. Unlike for *A. japonicus*, the present study uses sustainable source of *D. dentex* larvae from the Stella Mare hatchery in the context of an ecological restoration project (Ducos et al., 2022), and

it is relatively easy to produce sufficient quantities of these prey items to sustain a large-scale phyllosoma culture. The nutritional composition of *D. dentex* larvae could provide a useful guide on which to base future investigations for the definition of a formulated diet.

The present study found similar mortality patterns across all treatments, with a very low survival rate during the early phyllosoma stages, much higher survival during mid-larval stages, and a low survival rate from the final phyllosoma stages to the puerulus stage. The same trend has been reported by Kittaka and Kimura (1989) for Japanese spiny lobster (*Palinurus japonicus*) in regard to the early- and mid-phyllosoma stages. According to Kittaka and Kimura (1989), high mortality in the early stages of phyllosomas may be related to water quality deterioration, particularly the accumulation of organic matter derived from the feed provided to the larvae. This accumulation can cause fouling of the setae, thus impairing the swimming efficiency, increasing the energetic demand for swimming and lowering the oxygen uptake (Yeap et al., 2022). Given the high mortality observed from the final phyllosoma stages to the puerulus stage, there are a couple of possible explanations for these results: (i) the accumulation of opportunistic diseases throughout the six instars, the spreading of which can be managed by paying particular attention to hygiene in the rearing tanks. Disease could arise from an infection involving *Vibrio* spp. whose some strains are pathogenic leading to a variety of ‘vibriosis’ diseases (de Souza Valente and Wan, 2021), which induces symptoms such as cloudiness of the midgut gland that can cause a significant negative impact on animal production (Kittaka et al., 2001; de Souza Valente and Wan, 2021); and (ii) cessation of food intake during the beginning of the final phyllosoma stage before molting in the puerulus stage because of the retraction of the hepatopancreas (Fig. 5a, b) (Kittaka and Kimura, 1989, *Palinurus japonicus*, Yeap et al., 2022, *Palinurus elephas*). This cessation of feeding could highlight any nutritional deficiencies accumulated since the beginning of larval rearing. Indeed, during the larval development, the production of a new exoskeleton post-moult induces a decrease of the protein because during the intermoult periods, there is cessation of feeding (Yeap et al., 2022).

From the 435 pueruli produced at the end of the study (Fig. 6a), approximately 100 first stage juveniles were obtained (*i.e.*, 121 days after hatching or 10 days after metamorphosis) (Fig. 6b), 10 of which reached juvenile stage VII, corresponding to 335 days after hatching (*i.e.*, 224 days after metamorphosis) (Fig. 6c). In the literature, only one study (Kittaka et al., 2001) has obtained a *P. elephas* individual at a juvenile stage from a larval culture. This study maintained only one juvenile up to stage 9 (*i.e.*, 272 days after metamorphosis), while in another study, a juvenile individual captured in the wild was maintained up to stage 17 (*i.e.*, 756 days after metamorphosis) (Kanemaru, 1992).

**Table 3**  
Summary information for reared *Palinurus elephas* larvae.

References	Conditions			Larval progression				
	Temperature (°C)	Light intensity (lux)	Food source	Instars per tank	# of instars	Metamorphosis (Days)	# of pueruli	Survival rates (%) (± SE)
Present study	15 ± 0.5	26	<i>Dentex dentex</i> larvae	600	6	111	14	2.67 ± 1.20
		359		400*			164	41.50 ± 7.50
		910		600			257	42.00 ± 2.52
Kittaka et al. (2001)	19–20	–	<i>Arctoscopus japonicus</i> larvae	2000	6	65	2	0.1
		19–20		<i>Mytilus edulis</i> gonad			–	0
		16–18	Both foods	–	–	69	10	0.5
Kanemaru (1992)	–	–	–	–	6	149	0	0
Kittaka and Ikegami (1988)	17–20	–	<i>Artemia</i> nauplii, adult <i>Artemia</i> , and <i>Mytilus edulis</i>	5000	9	–	0	0
				10			0	0
				1000			0	0
				1000			0	0
				3000			1	0.03
		4000	–	0	0			

\* Only 400 individuals for light intensity 359 lux because of an abnormal peak of mortality observed for replicate 3 at 85 DAH; this sample was removed from the data set to avoid statistical bias.

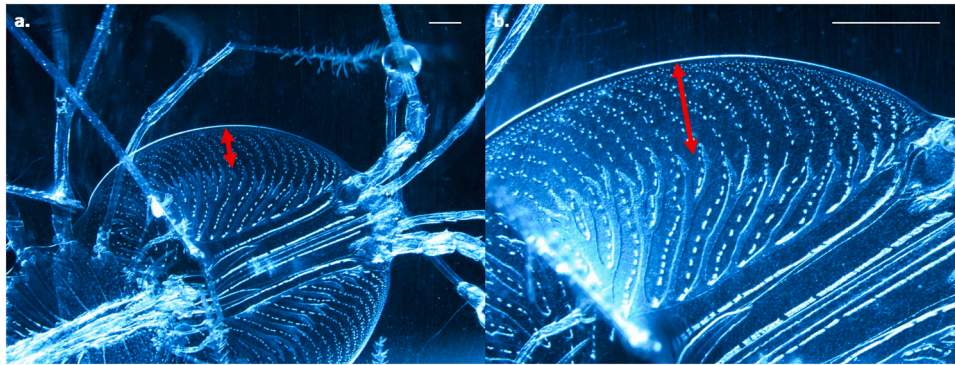


Fig. 5. *Palinurus elephas* phyllosoma stage VI (85 days after hatching) reared during the study. The red arrows show the gut retraction indicating the cessation of feeding. Scale bars in all images represent 1 mm.

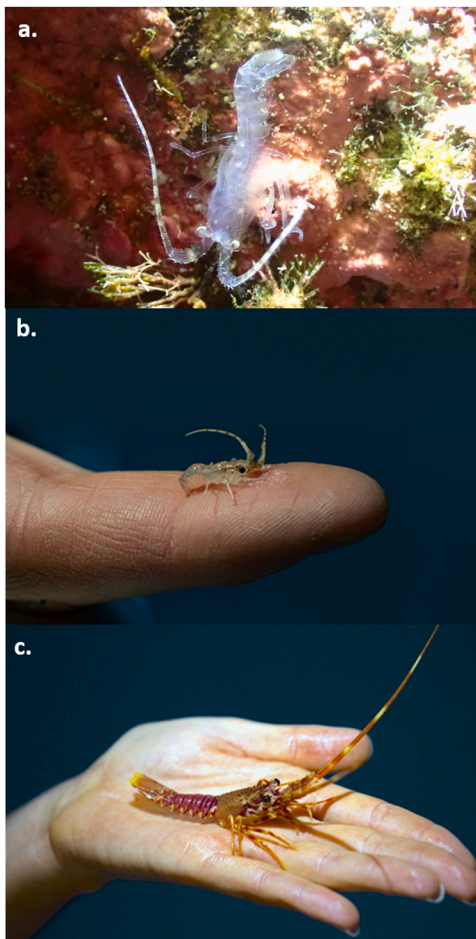


Fig. 6. *Palinurus elephas* puerulus and juvenile stages reared during the study; (a) Puerulus (111 days after hatching); (b) Juvenile stage I (121 days after hatching); (c) Juvenile stage VII (335 days after hatching).

Consequently, this study is the first study to rear a significant number of *P. elephas* juveniles.

## 5. Conclusion

The present study determined the rearing response of *Palinurus elephas* (European spiny lobster) larvae under different light intensities and found that the larval survival rate depended on the larval stage. Differences in light intensity did not affect the early stages but did influence

the final stages. Finally, the survival rate of phyllosomas and the metamorphosis to pueruli was substantially elevated in this study compared to previous studies. Nevertheless, further research is needed to identify the optimal light intensities, the light quality/spectrum, specifically the wavelength and evaluate the metabolic feeding efficiency of *P. elephas*.

This work has significant economic value, as it provides novel and essential knowledge for developing rearing protocols to improve larval performance and survival of *P. elephas*. Thus, findings of this study constitutes an important contribution for the exploitation of this species at commercial scale or to provide juveniles for experiments on the restoration of depleted populations.

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## CRediT authorship contribution statement

**Jean-José Filippi:** Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing – original draft. **Rémi Millot:** Conceptualization, Investigation, Data curation, Visualization, Writing – original draft, Writing – review and editing. **Jérémy Bracconi:** Writing – review and editing, Investigation, resources (*P. elephas* broodstock maintenance and larval rearing). **Viviana Ligorini:** Data curation, Writing – review and editing. **Nicolas Gattacceca, Ambre Crescioni, and Jean-Baptiste Ronchi-Perfetti:** Investigation, Resources (*P. elephas* broodstock maintenance and larval rearing). **Mikaël Demolliens, Sally Pugliese, Alban Delmas, and Alizée Boussard:** Resources (*D. dentex* broodstock maintenance and larval feed production). **Alexandre Vela:** Writing – review and editing. **Romain Bastien:** Project administration. **Vanina Pasqualini:** Writing – review and editing, Supervision. **Antoine Aiello:** Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2024.102083.

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