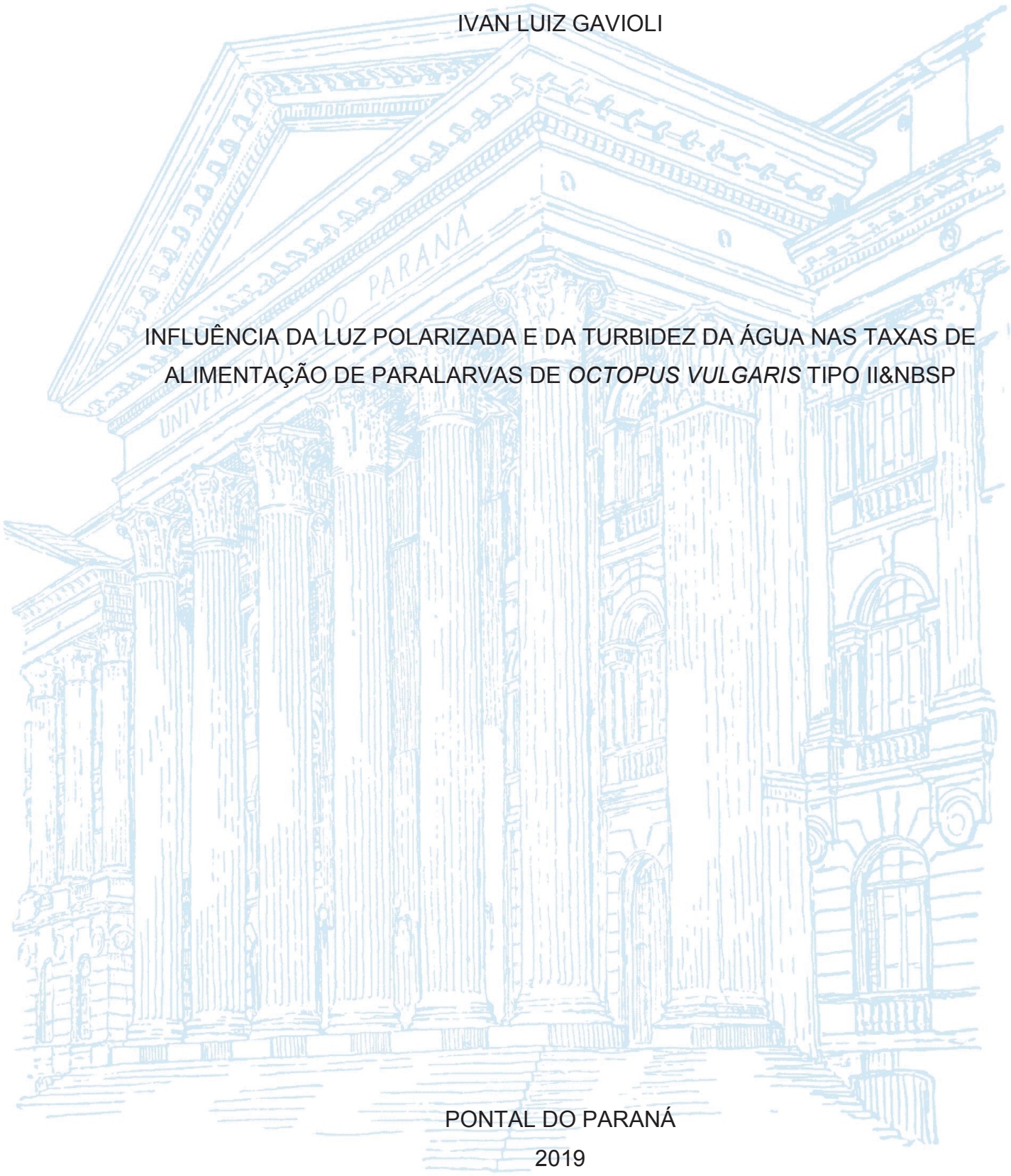
UNIVERSIDADE FEDERAL DO PARANÁ

IVAN LUIZ GAVIOLI



INFLUÊNCIA DA LUZ POLARIZADA E DA TURBIDEZ DA ÁGUA NAS TAXAS DE ALIMENTAÇÃO DE PARALARVAS DE *OCTOPUS VULGARIS* TIPO II

PONTAL DO PARANÁ

2019

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INFLUÊNCIA DA LUZ POLARIZADA E DA TURBIDEZ DA ÁGUA NAS TAXAS DE ALIMENTAÇÃO DE PARALARVAS DE *OCTOPUS VULGARIS* TIPO II

Dissertação apresentada ao curso de Pós-Graduação em Sistemas Costeiros e Oceânicos, Setor de Centro de Estudos do Mar, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Sistemas Costeiros e Oceânicos.

Orientadora: Profa. Dr. Erica Alves Gonzalez Vidal

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em SISTEMAS COSTEIROS E OCEÂNICOS da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **IVAN LUIZ GAVIOLI**, intitulada: **INFLUÊNCIA DA LUZ POLARIZADA E DA TURBIDEZ DA ÁGUA NAS TAXAS DE ALIMENTAÇÃO DE PARALARVAS DE *OCTOPUS VULGARIS* TIPO II **, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

A outorga do título de Mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 28 de Março de 2019.

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A dissertação que segue traz consigo não apenas o desenvolvimento de um conhecimento científico ou parte essencial de um título acadêmico. Ela representa, também, uma importante etapa na minha vida, visto que gera a grata sensação de que há um caminho profissional prazeroso a ser seguido. Este “encontrar-se” profissionalmente ocorreu ao mero acaso, sem qualquer plano bem arquitetado e executado de minha parte. Consequentemente, intensifiquei a minha visão pessoal de que nossos méritos nunca são exclusivamente nossos.

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RESUMO

O cultivo integral de polvos que produzem ovos pequenos tem como principal obstáculo as altas taxas de mortalidade e o baixo crescimento durante a fase de paralarva. Como as paralarvas de *Octopus vulgaris* são predadores visuais que demandam presa viva, hipotetizou-se que manipulando condições ambientais relacionadas à luz, as taxas de alimentação (TA) poderiam melhorar. Portanto, o objetivo deste estudo é avaliar se luz polarizada ou turbidez da água aumentam as TA de paralarvas de *O. vulgaris* recém eclodidas quando alimentadas com presa natural (copépode, *Acartia lilljeborgi*), bem como quantificar as TA e estimar o consumo energético diário (CED) das paralarvas. Uma paralarva recém-eclodida e 40 copépodes foram colocados em cada unidade experimental opaca (0.5 L, 9 cm de diâmetro, 13 cm de altura) contendo aeração suave por 24 h. Cinco réplicas foram utilizadas em cada experimento, junto com três controles. Estes continham apenas presas e foram utilizados para avaliar mortalidade natural e erros metodológicos tanto da manutenção quanto da quantificação das presas. O experimento de luz polarizada teve um Tratamento Controle (sem luz polarizada) e dois Tratamentos sob luz polarizada (com vetores eletrônicos a 90º e a 45º). O experimento de turbidez da água teve um Tratamento Controle (sem microalgas) e três outros Tratamentos com distintas densidades *de Isochrysis galbana* (5, 25 e 55 $x10^4$ cel. mL^{-1}). As TA diária das paralarvas foram quantificadas pela média obtida das diferenças entre o número de copépodes colocados no início do experimento e a quantidade de copépodes que sobraram em cada unidade experimental ao final do experimento. Uma TA geral foi estabelecida através da média da TA obtida em todos os tratamentos sem diferença significativa. O CED foi estimado através da multiplicação da TA observada pelo conteúdo energético individual dos copépodes. Não foram encontradas diferenças significativas nas taxas de alimentação nos experimentos de luz polarizada (p-valor 0.562) e de turbidez da água (p-valor 0.428). A variabilidade individual foi elevada com TA mínimas e máximas entre 1 a 10 copépodes $paralarva^{-1}$ dia^{-1} (luz polarizada) e de 0 a 7 copépodes $paralarva^{-1}$ dia^{-1} (turbidez da água). A taxa de alimentação geral foi de 3.86 ± 2.26 copépodes $paralarva^{-1}$ dia^{-1} e o CED médio foi de 0.135 cal. $paralarva^{-1}$ dia^{-1}. Este CED representa 99% da taxa metabólica de uma paralarva em repouso ou 18% de uma paralarva ativa. O que pode indicarque o metabolismo das paralarvas recém eclodidas depende principalmente da reserva vitelínica. Algumas hipóteses para explicar a falta de correlação entre TA e luz polarizada ou turbidez da água são apresentadas e discutidas. O valor de TA obtido usando presas naturais é um dado confiável e importante sobre as demandas diárias alimentares de paralarvas de *Octopus* em ambiente de cultivo. Esta informação será de grande valor para o estabelecimento de um protocolo alimentar para paralarvas desta espécie.

Palavras-chave: Cefalópodes. Predação. Resposta funcional. Iluminação. Sistema de água-verde. Taxa Metabólica.

ABSTRACT

The large scale culture of Octopuses that produce small eggs are mainly hindered by the high mortality and poor growth during the paralarval phase. Since *Octopus vulgaris* paralarvae are visual predators that requires live prey, we hypothesized that manipulating environmental conditions related to light could improve paralarvae feeding rates (FR). Therefore, the aim of this study is to evaluate if polarized light (PL) or water turbidity (WT) enhance newly-hatched *O. vulgaris* Type II paralarvae FR when fed on natural prey (copepods, *Acartia lilljeborgi*), as well as quantify their FR and estimate their daily energy consumption (DEC). Newly-hatched paralarva was placed together with 40 copepods into opaque black experimental units (0.5 L, 9 cm diameter, 13 cm height) with gently aeration for 24 h. Five replicate units were used for each treatment. Three Control replicates without paralarvae, but containing the prey, were used to evaluate natural mortality and methodological errors in prey maintenance and quantification. The PL experiment had a Control Treatment (no polarized light) and two under polarized light Treatments (the electronic vector at 90º and at 45º). The WT experiment had a Control Treatment (no algae) and three other Treatments with *Isochrysis galbana* indifferent densities (5, 25 and 55 $x10^4$ $cellsmL^{-1}$). Daily FR of paralarvae were quantified by the subtraction of the number of remaining copepods after the end of the experiment from the total number placed into each experimental unit. A general FR was established considering all non-significantly different treatments. DEC was estimated by multiplying the FR by the copepods individual energetic content. No significant differences on the FR were found for PL (p-value 0.562) and for WT (p-value 0.428). Individual variability was high with minimal and maximum FR ranging from 1to 10 copepods $paralarva^{-1}day^{-1}$, on the PL experiment and from 0 to 7 copepods $paralarva^{-1}day^{-1}$ on the WT experiment. The general FR was 3.86 ± 2.26 copepods $paralarva^{-1}day^{-1}$ and average DEC was 0.135 cal. $paralarva^{-1}day^{-1}$. The DEC value represented both 99% of metabolic rate (MR) of resting paralarvae and 18% of MR of active paralarvae. This could suggest that if the MR model used is not overestimated that paralarvae metabolism rely mainly on the yolk reserve. A range of hypotheses – from the sample size to the polarized vision development being dependent on stimuli and experience – are presented and discussed to explain the lack of correlation between FR and LP or WT. The FR values obtained using natural prey provide reliable and important data on the daily feeding requirements of *Octopus* paralarvae under culture conditions. This information will have special value in establishing a feeding protocol for rearing paralarvae.

Keywords: Cephalopods. Predation. Functional response. Green-water system, Illumination, Metabolic rate.

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INTRODUÇÃO GERAL

Polvos são moluscos cefalópodes, exclusivamente marinhos e ocupam uma ampla gama de habitats, desde regiões costeiras até o oceano profundo (Roper et al. 1984; Boletzky e Villanueva 2014). Possuem sistema nervoso e sensorial muito desenvolvido, respondem à estímulos mecânicos, químicos e visuais e são capazes de desempenhar tarefas complexas (Boletzky e Villanueva 2014). Este complexo sistema nervoso fez com que os cefalópodes fossem utilizados por mais de um século como modelos experimentais em neurobiologia e fisiologia (Fiorito et al. 2014, 2015). Avanços nesta área de estudo levaram à identificação de que cefalópodes sentem dor, sofrimento e angústia (Andrews et al. 2013), o que os tornou a primeira classe de invertebrados a ser enquadrada na diretiva europeia que regula o uso, bem-estar e proteção de animais utilizados para fins científicos (Directive 2010/63/EU).

O polvo comum – *Octopusvulgaris*, Cuvier 1797 – é considerado a espécie tipo representante do gênero *Octopus* e foi historicamente considerada cosmopolita (Guerra 1997), com ampla distribuição geográfica em águas tropicais, subtropicais e temperadas. Entretanto, estudos filogenéticos e moleculares recentes demonstram que esta espécie é polifilética, derivando de mais de um ancestral evolutivo (Amor et al. 2017). Assim, *Octopus vulgaris* abrange, na realidade, um complexo de espécies geneticamente distintas denominadas como "*Octopus vulgaris* complexo de espécies" (Amor et al. 2017). A distinção entre as espécies deste complexo foi definida conforme isolamento geográfico e ausência de fluxo gênico plausível (Norman et al. 2014; Fig. 1). O complexo de espécies é formado por *Octopus vulgaris sensu stricto (ss)*, localizado no mediterrâneo e Atlântico Norte Oriental e várias espécies Tipo. Entre elas o Tipo I, ocorrendo no Caribe e Golfo do México; o Tipo II, na costa brasileira; o Tipo III, ao longo da costa sul-africana nos oceanos Atlântico e Índico; e o *Octopus sinensis*, nas águas subtropicais e temperadas da Ásia Oriental (Mares do Japão, da China Oriental e Oceania) (Amor et al. 2017).

Todas as espécies do complexo apresentam três fases distintas ao longo do ciclo de vida: paralarva, juvenil ou sub-adulta e adulta (Boletzky e Villanueva 2014). Após a cópula, a fêmea mantém os espermatóforos do(s) macho(s) dentro de seu oviduto por semanas/meses até que encontre condições ideais para fecundar seus óvulos (Quintero et al. 2011). Após a postura dos ovos a fêmea reduz a alimentação, chegando a cessar por completo a ingestão de alimentos (Nesis 1995), e permanece limpando e aerando os milhares de ovos até o fim de sua vida (Boletzky e Villanueva 2014; Iglesias e Fuentes 2014). O período embrionário dura cerca de 38 dias a 18 ºC (Iglesias e Fuentes 2014) e seu sucesso em sistemas de cultivo – mensurado pela eclosão de paralarvas de qualidade – depende da qualidade nutritiva da dieta da fêmea anteriormente à fecundação (Márquez et al. 2013; Quintana

et al. 2015), da permanência da fêmea junto aos ovos (Iglesias e Fuentes 2014) e da temperatura da água (Boletzky 1987). Quando o desenvolvimento embrionário ocorre em temperaturas mais elevadas, as paralarvas produzidas são menores, com reduzida reserva vitelínica nos primeiros dias de vida (Vidal et al. 2002). Para eclodir, os polvos se utilizam de enzimas produzidas por uma glândula especial na extremidade do manto, as quais digerem o córion do próprio ovo para que o embrião possa deixar o ovo e ecloda como paralarva (Boletzky e Villanueva 2014).

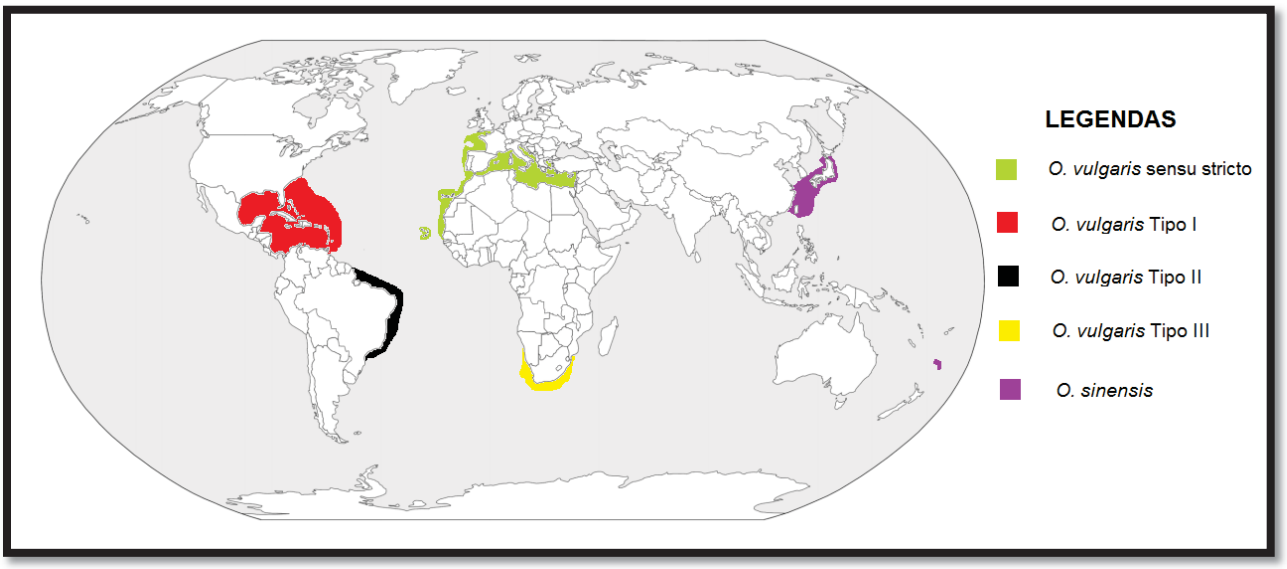

Figura 1– Distribuição geográfica das espécies do *Octopus vulgaris* complexo de espécies (mapa produzido pelo Autor baseado em Amor et al. 2017).

O termo paralarva – ou falsa larva – foi cunhado por Young e Harman (1988) para definir aqueles cefalópodes que apresentam desenvolvimento embrionário direto, porém com habitat, ecologia e comportamento distintos dos adultos coespecíficos. As paralarvas são planctônicas ao eclodir, apresentando natação ativa e alta taxa metabólica (Iglesias et al. 2007, 2014), a qual é suprida inicialmente pela reserva vitelínica e, posteriormente, por uma alimentação carnívora voraz (Boletzky e Villanueva 2014). Ao longo da fase planctônica, que dura em torno de 47-54 dias a 21.2ºC, ocorrem mudanças morfológicas como alongamento dos braços, aumento do número de ventosas (de 3 para 17-20 por braço), surgimento de cromatóforos, iriodóforos e leucóforos, perda dos órgãos de Kölliker e da primeira dentição do bico (Boletzky e Villanueva 2014; Iglesias e Fuentes 2014; Franco-Santos et al. 2014). Ao final da fase planctônica, os polvos assentam e iniciam o período juvenil ou sub-adulto com modo de vida bentônico, ocupando costões rochosos e recifes de coral (Mangold 1983), desde que o ambiente apresente condições favoráveis como abrigo e alimento (Mather e O'Dor 1991). Durante a fase juvenil os polvos apresentam taxas de crescimento exponencial, supridas pelo consumo

de variadas espécies bentônicas (Mather e O'Dor 1991). A fase adulta se inicia com a maturação sexual dos organismos após a liberação pela glândula óptica de hormônio gonadotrófico (Greenwell 2017). Quando adulto, o polvo comum apresenta pouco dimorfismo sexual (Boletzky e Villanueva 2014), comportamento não sociável (Ikeda 2009; Scheel et al. 2016; Edsinger e Dölen 2018) e apenas um ciclo reprodutivo (Nesis 1995).

Atualmente, o condicionamento e engorda de juvenis e posterior manutenção de adultos – bem como a cópula e reprodução – são facilmente obtidas em cativeiro se ofertadas condições ambientais ideais, abrigo e alimentação adequada (Iglesias e Fuentes 2014). Comercialmente, apesar de eventualmente ocorrer canibalismo (Ibánez e Keyl 2013) e crescimento desigual entre indivíduos devido a questões hierárquicas e territorialistas (Boletzky e Villanueva 2014), a engorda em escala comercial de *Octopus vulgaris* é realizada com sucesso na Espanha a partir de juvenis selvagens mantidos em estruturas no mar e alimentados com espécies de crustáceos, peixes e moluscos de baixo valor comercial (García et al. 2014).

A engorda de polvos visa suprir a demanda para consumo humano, apostando no elevado valor comercial da carne (Garcia et al. 2014), no mercado consumidor amplo e em expansão (Doubleday et al. 2016; Globefish 2019). Além destes aspectos socioeconômicos, diversas características zootécnicas favoráveis tornaram o *O. vulgaris* complexo de espécies um potencial candidato à aquicultura nas últimas décadas. Os polvos deste complexo de espécies representam ótima fonte proteica, com cerca de 84% de seu peso seco sendo formado por proteínas, e tem um rendimento de carcaça de 80-85% do corpo inteiro (Lee 1994). Além disso, possuem ciclo de vida curto e alta fecundidade com a produção de centenas de milhares de ovos por fêmea e incorporam de 40-60% do alimento ingerido, apresentando crescimento de até 3% ao dia (Vaz-Pires et al. 2004; Iglesias et al. 2007; Iglesias & Fuentes 2014; Vidal et al. 2014).

Apesar do alto desempenho zootécnico, existem obstáculos que retardam o desenvolvimento da atividade. Dentre eles destacam-se: a alta demanda proteica durante todo o ciclo de vida (Iglesias et al. 2004; Domingues et al. 2007; Uriarte et al. 2014); a elevada demanda por ácidos graxos durante as fases embrionária, paralarval e juvenil (Navarro e Villanueva 2000; Iglesias et al. 2007; Seixas et al. 2010); e a elevada exigência de qualidade da água durante a larvicultura (Villanueva e Norman 2008). O principal gargalo técnico-científico da atividade, inclusive, encontra-se na larvicultura, devido às altas taxas de mortalidade e baixo crescimento das paralarvas (Garrido et al. 2016).

A nutrição tem sido apontada como uma das principais causas do baixo crescimento e sobrevivência nesta etapa de cultivo (Villanueva 1994, 1995; Moxica et al. 2002; Vidal et al. 2002; Iglesias et al. 2004, 2007, 2014; Carrasco et al. 2005; Boletzky e Villanueva 2014) visto que as paralarvas demandam grande quantidade de alimento vivo com qualidade nutricional elevada. Visando

superar este gargalo técnico-científico para o cultivo integral de *O. vulgaris*, vários pesquisadores têm estudado a nutrição das paralarvas, investigando os efeitos de diversos nutrientes como ácidos graxos (Navarro e Villanueva 2000; Seixas et al. 2010; Garrido et al. 2016; Monroig et al. 2017), aminoácidos (Villanueva et al. 2004), micronutrientes essenciais e não-essenciais (Villanueva e Bustamante 2006; Villanueva e Norman 2008) e relação proteína/lipídios (Seixas et al. 2010). Consequentemente, muitos avanços foram feitos ao se identificar os benefícios oriundos de uma dieta com elevado conteúdo proteico e de ácido docosaexaenoico (DHA) (Navarro e Villanueva 2000, 2003; Seixas et al. 2010; Guinot et al. 2013; Garrido et al. 2016), da lecitina marinha (Morales et al. 2017), do cobre (Villanueva e Bustamante 2006; Villanueva et al. 2017) e também a preferência das paralarvas de *O. vulgaris sensu stricto* por larvas de crustáceos (zoeae) decápodes na natureza (Roura et al. 2012, 2016; Olmos- Pérez et al. 2017).

Paralarvas de *O. vulgaris* apresentam melhores taxas de crescimento e de sobrevivência quando são alimentadas com zoeae de decápodes do que quando alimentadas com *Artemia* (Iglesias et al. 2007, 2014; Garrido et al. 2017; Dan et al. 2018; Perales-Raya et al. 2018). As principais razões para isto estão relacionadas ao fato de que presas naturais de cefalópodes como misidáceos, zoeae de decápodes e copépodes marinhos (Drillet et al. 2006; Roura et al. 2012) são ricos em ácidos graxos poli-insaturados n-3 e tem uma proporção entre ácido docosaexaenoico (DHA) e ácido eicosapentaenoico (EPA) de 1:1 (Navarro e Villanueva 2000, 2003). Já a *Artemia* – alimento vivo mais utilizado para larvicultura de peixes marinhos e crustáceos (Sorgeloos et al. 2001) – apresenta baixa quantidade de ácidos graxos poli-insaturados n-3, e metaboliza o DHA (essencial para as paralarvas) em EPA (Navarro et al. 1999; Navarro e Villanueva 2000; Reis et al. 2017). Além disso, *Artemia* apresenta baixo conteúdo em cobre (Villanueva e Bustamante 2006), baixa quantidade de fosfolipídios (García-Garrido et al. 2010; Reis et al. 2019) e alto teor de triglicerídeos (Reis et al. 2019). Deste modo, somente Moxica et al. (2006) e De Wolf et al. (2011) obtiveram sucesso no cultivo de paralarvas de *O. vulgaris* ao utilizar *Artemia* enriquecida – porém as taxas de crescimento (publicadas somente por Moxica et al. 2006) foram menores do que em cultivos bem-sucedidos que utilizaram zoeae de decápodes.

A utilização de zoeae de decápodes só é atualmente exequível em cultivos experimentais, sendo inviável em escala comercial devido aos custos e às dificuldades logísticas para produção de zoeae (Martín 2017; Reis et al. 2019). Além disso, experimentalmente, as taxas de sobrevivência de paralarvas de *O. vulgaris* até o assentamento tem variado entre 0 e 31.5% quando se utiliza zoeae como presa (Villanueva 1995; Iglesias et al. 2004; Carrasco et al. 2006; Garrido et al. 2016). Estes valores ainda estão longe do ideal e deste modo ainda se avalia a realização do cultivo de paralarvas de *O. vulgaris* utilizando apenas *Artemia* (Villanueva et al. 2014; Reis et al. 2019) ou presas alternativas

(Iglesias et al. 2007) cuja produção em escala comercial seja possível e economicamente viável. O uso de presas alternativas e variadas é favorável visto que o conteúdo microbiano do sistema digestivo das paralarvas é mais rico em ambiente natural do que quando alimentado apenas com *Artemia* (Roura et al. 2017).

Os copépodes estão entre os metazoários mais numerosos (Lee et al. 2010), sendo abundantes em regiões que servem de berçário para espécies marinhas (Chesney 2005). Além disso, são costumeiramente utilizados em pesquisas na aquicultura como alimento vivo (Dahms et al. 2006), apresentam maior valor nutricional do que rotíferos e *Artemia* (Sargent et al. 1997; Iglesias et al. 2007) e são mais facilmente produzidos em larga escala (Naas et al. 1991; Bersano 2003) do que as zoeae (Zmora et al. 2005). Além disso, apesar da preferência das paralarvas de *O. vulgaris* por larvas de crustáceos decápodes (Roura et al. 2012, 2016; Olmos- Pérez et al. 2017), copépodes são comumente predados em ambiente natural (Olmos-Pérez et al. 2017) e eficientemente ingeridos e digeridos por paralarvas em cultivo (Nande et al. 2017).Entretanto, a captura de copépodes por paralarvas pode ser um desafio devido a movimentação errática e rápida resposta de fuga da presa. Assim, compreender fatores que influenciam a captura de presas são essenciais para o desenvolvimento do cultivo de paralarvas, já que estas são predadoras vorazes que requerem elevada quantidade de alimento vivo de qualidade para suprir seu elevado metabolismo (Villanueva e Norman 2008).

Similarmente ao descrito por Messenger (1968) em *Sepia officinalis*, Hernández-Garcia et al. (2000) identificaram que paralarvas de *Octopus vulgaris* apresentam três fases no processo de predação: atenção, posicionamento e ataque. Na primeira fase, a paralarva reduz sua velocidade, mantendo a natação com deslocamentos mais curtos que o usual e próximos da presa selecionada (Hernández-Garcia et al. 2000). Em seguida, a paralarva se posiciona com a ponta dos braços apontados diretamente para a presa, quando então executa o ataque com um movimento para a frente em direção ao alvo (Hernández-Garcia et al. 2000; Villanueva et al. 1996). Após a captura, a presa é manipulada pela paralarva, a qual não cessa sua natação (Hernández-Garcia et al. 2000). Uma vez que a presa tenha sido selecionada, as distâncias de ataque geralmente são de duas a quatro vezes o comprimento total da paralarva. Para paralarvas de *O. vulgaris* de 30 dias de vida (7,4 mm de comprimento total), a distância média de reação máxima (R) foi de 15,5± 9.42 mm (Villanueva et al. 1996). Deslocamentos para a frente são sempre usados para capturar presas, enquanto deslocamentos para trás são utilizados enquanto a paralarva domina, manipula e ingere a presa (Villanueva et al. 1996). O tempo transcorrido entre o ataque à presa e a natação para trás é de 0,3 s (Villanueva et al. 1996). Quando o ataque em direção a uma presa falha, a paralarva repete o movimento em até 2 s, chegando a executar até três ataques consecutivos.

Apesar da importância ecológica e comercial de *Octopus vulgaris,* informações sobre a taxa de alimentação (quantidade de presas consumidas por um predador em um determinado período de tempo – Hassell 1978) das paralarvas é pouco conhecida. Itami et al. (1963) identificou que paralarvas de *O. sinensis* com comprimento total de 3-5 e 6-8 mm consumiram, respectivamente, 3-5 e 7-10 zoeae de *Palaemon serrifer* (tamanho médio de 2-3 mm) por dia a 25 ºC. Márquez et al. (2007) identificou que paralarvas de *Octopus vulgaris* recém-eclodidas a 20 ºC tem uma taxa alimentar de 0.8-16 metanáuplios de *Artemia* (0,8 mm). Garrido et al. (2016) por sua vez, obteve taxas média de alimentação de 31 e 15,4 quando as presas eram *Artemia* e *Tisbe*, respectivamente. Estes valores foram obtidos a 22,6 ºC, intensidade luminosa de 100 lux e densidade de presas de 1000 presas.L^{-1}.

O sucesso do processo predatório depende de fatores diversos como a percepção da presa pelo predador, o tamanho, a morfologia, a mobilidade, a pigmentação, o comportamento e a palatabilidade da presa, bem como a capacidade natatória e a capacidade de aprendizado do predador, além de fatores ambientais (Chesney 2005; Vidal e Boletzky 2014). Como as paralarvas de *Octopus vulgaris* são predadores táteis-visuais (Márquez et al. 2007; Villanueva e Norman 2008; Boletzky e Villanueva 2014; Vidal e Boletzky 2014) e o desenvolvimento precoce da visão é essencial para aumentar as chances de sobrevivência (Darmaillacq et al.2017), a luz é um fator abiótico crucial no processo predatório (Márquez et al. 2007; Sykes et al. 2011; Villanueva et al. 2017).

A luz sofre mudanças assim que penetra na água. As partículas presentes na coluna da água e as próprias moléculas da água absorvem e dispersam a luz de forma seletiva, afetando a intensidade, o comprimento de onda, a direção, a propagação, a polarização e o espectro (Garrido et al. 2017). A importância do espectro luminoso para larvas de peixes marinhos já é bem conhecida visto que peixes apresentam mais de um pigmento em suas retinas (Valen et al. 2014). Para os cefalópodes, entretanto, os quais são evidencialmente classificados como incapazes de distinguir cores (Messenger 1977; Mähthger et al. 2009) por conterem apenas um pigmento em suas retinas (Chung e Marshall 2017), a influência do espectro ainda não é tão bem compreendido. Tanto que, apesar de apresentarem apenas um pigmento, os polvos são mestres da camuflagem no ambiente marinho. Stubbs e Stubbs (2016) sugerem que apesar de cefalópodes conterem apenas um pigmento reticular, também apresentam um mecanismo para identificar cores através de aberrações cromáticas e de pupilas fora do eixo, permitindo focar em diferentes espectros de onda luminosa.

Estudos recentes demonstraram que paralarvas de *O. vulgaris* apresentaram preferência por espectro luminoso branco e azul ao invés de verde e vermelho (Martín 2017), conforme esperado, visto que o pigmento da retina do *O. vulgaris* apresenta absorção máxima na região azul do espectro luminoso (Chung e Marshall 2017). Em termos de sobrevivência e crescimento, Martín (2017) identificou que luzes azul e branca aumentaram as taxas de sobrevivência de paralarvas de *O. vulgaris*,

mas não alteraram o crescimento. Por outro lado, Tur et al. (2018) ao testarem a influência do espectro luminoso na sobrevivência e crescimento de paralarvas da mesma espécie Tipo, identificaram apenas diferenças no crescimento, mas não na sobrevivência. No que tange a predação, a utilização de luzes branca, verde e azul resultaram em maiores taxas de alimentação do que a luz vermelha (Martín 2017). Portanto, é bem possível que esta variação nas taxas de alimentação, sobrevivência e crescimento estejam relacionadas a outras características da luz ainda pouco compreendidas.

Em larviculturas de *O. vulgaris* realizadas em tanques sombreados e naqueles em que a luz penetra obliquamente na coluna da água, as taxas de sobrevivência foram maiores do que em tanques sem sombreamento e em tanques em que a luz penetra verticalmente na coluna da água (Tur et al. 2018). Entretanto, ao testar concomitantemente a luz oblíqua em tanque sombreado não houve aumento nas taxas de sobrevivência em comparação com a utilização individual de luz oblíqua ou tanque sombreado. Os autores sugerem que este resultado possa estar relacionado com o fato de que tanto a luz oblíqua quanto o tanque sombreado geram um ambiente com maior variedade de intensidade luminosa. Estas condições imitam o ambiente natural e a migração vertical das paralarvas, as quais ocupam a superfície à noite e regiões mais profundas durante o dia (Roura et al. 2016, 2019; Olmos-Pérez et al. 2017).

A importância da intensidade luminosa como fator chave para o sucesso na predação pelas paralarvas está cada vez melhor documentada. A taxa de predação de *Artemia* por paralarvas de *O. vulgaris* recém-eclodidas é três vezes maior na presença de luz do que na sua ausência (Márquezet al. 2007) e as taxas de sobrevivência e de crescimento aumentam na medida em que e eleva a intensidade luminosa entre 10 e 600 lx (Tur et al. 2018).

A polarização da luz é outra característica luminosa importante. A luz natural, ao ser dispersa pelas moléculas da água – ou ao passar por um filtro dicroico – polariza-se. Ou seja, a vibração da luz que emana do sol ocorrendo em todas as direções é filtrada, passando a vibrar em uma única direção (Marshall e Cronin 2011). A direção da vibração – a qual difere da direção de propagação – é comumente chamada de vetor eletrônico da luz (e-vetor). Deste modo, a luz polarizada é a luz cujo e-vetor vibra em apenas uma direção específica. Estudos em cefalópodes adultos (e.g. *O. vulgaris* e *O. briareus*, Shashar e Cronin 1996; *Doryteuthis pealei* e *Euprymna scolopes*, Shashar e Hanlon 1996; *Sepia officinalis*, Shashar et al. 2000) demonstraram que estes animais além de serem sensíveis à luz polarizada, também são capazes de reconhecer e atacar objetos cuja luz foi polarizada. Ou seja, eles não só possuem a habilidade de perceber diferentes orientações do vetor, normalmente utilizada para orientação, mas também demonstraram ser capazes de identificar alvos cuja incidência de luz está linearmente polarizada em um mesmo e-vetor.

Paralarvas de *Doryteuthis pealei* são capazes de efetuar ataques em presas que estão 70% mais distantes quando há luz polarizada do que na ausência desta (Shashar et al. 1998). Consequentemente, sugere-se que é possível melhorar as taxas de alimentação das paralarvas utilizando-se a luz polarizada, o que aumentaria as taxas de alimentação e sobrevivência durante a larvicultura (Villanueva et al. 1996; Megrey e Hinckley 2001; Chesney 2005; Vidal et al. 2014). Entretanto, em estudos comparalarvas de *O. vulgaris* alimentadas com *Artemia* ou o copépode bentônico *Tisbe* sp.como presa na presença de luz polarizadanão foram observados aumentos na taxa de predação (Garrido et al. 2016; Martín 2017). As justificativas apresentadas para explicar este feito foram baseadas na facilidade das paralarvas em localizar suas presas em ambiente confinado (Garrido et al. 2017) e pelo fato de serem paralarvas recém eclodidas (Martín 2017). Além disso, todos os experimentos foram feitos durante o período de vida em que as paralarvas ocupam águas superficiais costeiras, antes de serem transportadas por correntes para águas oceânicas e mais profundas (Roura et al. 2016, 2019), onde a polarização pode apresentar maior influência. Outra explicação possível é que as presas ofertadas sejam as responsáveis pelos resultados, já que tanto *Artemia* como *Tisbe* apresentam natação lenta, facilitando sua predação.

A escolha de presas para testar a luz polarizada é essencial, visto que elas também apresentam um papel importante na geração do contraste entre elas e o meio aquático (Shashar et al. 1998). Muitos organismos planctônicos são transparentes para dificultar a sua visualização em ambientes aquáticos, através da camuflagem do seu brilho. Este processo, entretanto, resulta na polarização parcialmente linearizada da luz refletida de suas epidermes (Denton 1970; Rowe e Denton 1977 *apud* Shashar et al. 2000; Cartron et al. 2013). Esta luz, já polarizada, chega até os olhos dos cefalópodes, cujas microvilosidades próximas das células fotorreceptoras presentes na retina estão distribuídas ortogonalmente, o que garante a habilidade de escolher e atacar alvos que reflitam a luz polarizada (Shashar et al. 2000; Cartron et al. 2013).

Além da luz polarizada, outro fator capaz de ampliar o contraste da presa com o ambiente é a turbidez da água (De Wolf et al. 2011; Vidal e Boletzky 2014). A turbidez da água pode ser alterada pela abundância de partículas suspensas e de microalgas ao interferir na luz que penetra na coluna d'água. Enquanto partículas em suspensão podem ser deletérias nos cultivos aquícolas (Timmons e Ebeling 2002; Vidal e Boletzky 2014), os efeitos da utilização de microalgas nos tanques de cultivo de peixes marinhos e cefalópodes parecem serem favoráveis ao cultivo. Em muitos casos os cultivos em água verde – como são conhecidos os cultivos em que microalgas são adicionadas aos tanques – favorecem a estabilização da qualidade da água por removerem os compostos nitrogenados e aportarem oxigênio, mantendo um controle microbiótico do meio ao criar competição apossíveis patógenos, serem fontes indiretas de nutrientes quando se utiliza alimentos vivos e por favorecerem o

contraste visual (Lavens e Sorgeloos 1996; Naas et al. 1996; Planas e Cunha 1999; Timmons e Ebeling 2002).

Com base no exposto acima, a relevância da turbidez da água e da polarização da luz como fatores abióticos que podem influenciar as taxas de alimentação em paralarvas de *O. vulgaris* fica evidente. Apesar do uso de cultivos em água verde ser comumente utilizado em cultivos de paralarvas e larvas de peixes marinhos, não identificamos estudos que tenham mensurado o impacto desta técnica nas taxas de alimentação das paralarvas. No que tange à luz polarizada, estudos com paralarvas de lula foram bem-sucedidos ao demonstrar que as paralarvas já apresentam visão polarizada desenvolvida (Shashar et al. 1998) assim como os adultos coespecíficos (Shashar e Hanlon 1996), entretanto, não há evidências até o momento de que isso possa favorecer o sucesso de predação. Assim, o presente estudo visa quantificar o sucesso predatório (através da taxa de alimentação) com presas naturais que apresentam um melhor perfil nutricional e que podem ser produzidas em maiores escalas.

Objetivos

Os objetivos deste estudo são: avaliar se luz artificialmente polarizada ou turbidez da água aumentam a taxa de alimentação de paralarvas de *Octopus vulgaris* Tipo II recém eclodidas quando alimentadas com alimento natural (*Acartia lilljeborgi*); bem como quantificar as taxas de alimentação de paralarvas para determinar o consumo energético das mesmas.

Hipóteses

Isso porque, como paralarvas de *O. vulgaris* Tipo II são predadores visuais que requerem presas vivas, ao manipularmos condições ambientais como a incidência de luz polarizada e turbidez da água, podemos favorecer a predação das paralarvas, resultando em uma maior taxa de alimentação. Ao facilitar a predação de presas naturais de elevado valor nutricional, podemos melhorar as taxas de crescimento e sobrevivência das paralarvas no período mais crítico do cultivo.

Revista pretendida

O artigo abaixo foi redigido conforme as normas da revista **Aquaculture**, visto que a pesquisa se enquadra na seção “nutrição” da revista por apresentar resultados inovadores a respeito da

optimização de práticas de alimentação para cultivos aquícolas. Além disso, a maior parte dos estudos com que este estudo dialoga estão presentes em publicações anteriores deste jornal cujo fator de impacto (2017) é de 2,710, com *CiteScore* de 3,05 e SNIP (2017) de 1,580.

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CONNECTING POLARIZED LIGHT AND WATER TURBIDITY WITH FEEDING RATES IN *Octopus vulgaris* Type II PARALARVAE

ABSTRACT

The large scale culture of Octopuses that produce small eggs are mainly hindered by the high mortality and poor growth during the paralarval phase. Since *Octopus vulgaris* paralarvae are visual predators that requires live prey, we hypothesized that manipulating environmental conditions related to light could improve paralarvae feeding rates (FR). Therefore, the aim of this study is to evaluate if polarized light (PL) or water turbidity (WT) enhance newly-hatched *O. vulgaris* Type II paralarvae FR when fed on natural prey (copepods, *Acartia lilljeborgi*), as well as quantify their FR and estimate their daily energy consumption (DEC). Newly-hatched paralarva was placed together with 40 copepods into opaque black experimental units (0.5 L, 9 cm diameter, 13 cm height) with gently aeration for 24 h. Five replicate units were used for each treatment. Three Control replicates without paralarvae, but containing the prey, were used to evaluate natural mortality and methodological errors in prey maintenance and quantification. The PL experiment had a Control Treatment (no polarized light) and two under polarized light Treatments (the electronic vector at 90º and at 45º). The WT experiment had a Control Treatment (no algae) and three other Treatments with *Isochrysis galbana* indifferent densities (5, 25 and 55 $x10^4$ $cellsmL^{-1}$). Daily FR of paralarvae were quantified by the subtraction of the number of remaining copepods after the end of the experiment from the total number placed into each experimental unit. A general FR was established considering all non-significantly different treatments. DEC was estimated by multiplying the FR by the copepods individual energetic content. No significant differences on the FR were found for PL (p-value 0.562) and for WT (p-value 0.428). Individual variability was high with minimal and maximum FR ranging from 1 to 10 copepods $paralarva^{-1}day^{-1}$, on the PL experiment and from 0 to 7 copepods $paralarva^{-1}day^{-1}$ on the WT experiment. The general FR was 3.86 ± 2.26 copepods $paralarva^{-1}day^{-1}$ and average DEC was 0.135 cal. $paralarva^{-1}day^{-1}$. The DEC value represented both 99% of metabolic rate (MR) of resting paralarvae and 18% of MR of active paralarvae. This could suggest that if the MR model used is not overestimated that paralarvae metabolism rely mainly on the yolk reserve. A range of hypotheses – from the sample size to the polarized vision development being dependent on stimuli and experience – are presented and discussed to explain the lack of correlation between FR and LP or WT. The FR values obtained using natural prey provide reliable and important data on the daily feeding requirements of *Octopus* paralarvae under culture conditions. This information will have special value in establishing a feeding protocol for rearing paralarvae.

Keywords: Cephalopods. Predation. Functional response. Green-water system, Illumination, Metabolic rate.

Introduction

The common octopus – *Octopus vulgaris*, Cuvier 1797 – is one of the most studied cephalopods worldwide (Iglesias et al. 2007; Vidal et al. 2014) due to its ecological and fishery significance, for being model for neurobiology and neuroscience studies and its wide distribution (Villanueva and Norman 2008; Iglesias and Fuentes 2014). Despite being considered a cosmopolitan species (Guerra 1997), it has been demonstrated that *Octopus vulgaris* comprise a complex of genetically distinct species known as "*Octopus vulgaris* species complex" (Amor et al. 2017). Many species of the *O. vulgaris* complex have biological features that qualify them as promising candidates for aquaculture. Some features stand out as the short life cycle, high fecundity, elevated food conversion rate, fast growth, high protein content and established consumer markets (Vaz-Pires et al. 2004; Iglesias et al. 2007; Iglesias and Fuentes 2014; Vidal et al. 2014, Globefish, 2019). Besides these attractive features, massive mortality and poor growth commonly recorded during the planktonic paralarval phase under laboratory conditions currently constrain commercial culture of *Octopus vulgaris* (Iglesias et al. 2007; Vidal et al. 2014). Several studies worldwide suggest that this bottleneck is caused by nutritional factors and lack of well-defined rearing conditions and protocols during this phase (Iglesias et al. 2007; Villanueva et al. 2009; Navarro et al. 2014; Iglesias and Fuentes 2014; Garrido et al. 2016;Olmos-Pérez et al. 2017).

Light is one of the most important factors for successful rearing of octopus paralarvae since it is a key environmental factor that synchronizes all life-stages from embryo development to sexual maturation (Villamizar et al. 2011). Light influences on paralarvae development according to the photoperiod (Iglesias et al. 2007), intensity (Márquez et al. 2007a) and spectrum (Martín 2017, Tur et al. 2018). However, light polarization and water absorbance properties have not been scrutinized so far despite some initial effort by Garrido et al. (2016) and Martín (2017) regarding polarization. Thus, it would be important to evaluate both the influence of polarized light and water turbidity on paralarvae feeding rates, since an increment on the feeding rates could directly improve survival and growth rates under culture conditions (Morales et al. 2017, Varo et al. 2017). In addition, feeding rates allows identifying the amount of energy ingested by the predator, enabling to identify their energy balance.

Feeding rates hinge to successful predation, which depends on a variety of factors, such as size, morphology, mobility, pigmentation, behavior, and palatability of the prey, as well as the swimming and escape capacity of the predator, besides environmental factors (Chesney 2005; Vidal and Boletzky 2014). Light is a key environmental factor and has been proved to impact foraging, growth, and survival in marine larvae (Monk et al. 2006; Villamizar et al. 2009) and in *Octopus vulgaris* paralarvae

(Martín 2017). Considering that *O. vulgaris* paralarvae are a tactile-visual predator (Márquez et al. 2007a, Villanueva and Norman 2008), light intensity and wavelength (Márquez et al. 2007a, Martín 2017), as well as light polarization (Shashar et al. 1998), should play important roles on predation.

Light polarization happens when a beam of light is scattered by water molecules or passes through linearly polarizing dichroic filters. On these occasions some angles of vibration – also known as electronic vector (e-vector) – are absorbed by the filter, making light vibrate in a single plane and not to all directions (Marshall and Cronin 2011). It has been shown that some adult cephalopods are capable of choosing and attacking targets when there is polarized light (Shashar and Cronin 1996; Shashar and Hanlon 1996; Shashar et al. 2000). Squid paralarvae (*Doryteuthis pealei*), for example, are able of attacking prey that are 70% farther when artificial polarized light is offered than when it is absent (Shashar et al. 1998). Even though the visual acuity of *O. vulgaris* paralarvae is poorly understood, it is plausible to suppose that they have polarized vision as other cephalopods, what should help in the perception of transparent and translucent prey (Shashar et al. 1998, 2000).

Water turbidity is another light related factor as it influences light penetration, enhancing underwater contrast (Lavens and Sorgeloos 1996; Timmons and Ebeling 2002) and, thus, influencing predation (Chesney, 2005; Villanueva and Norman, 2008; De Wolf et al., 2011; Vidal and Boletzky, 2014). Water turbidity can be understood as the measure of the scattering of light produced by the presence of suspended or colloidal particles, being expressed as Nephelometric Turbidity Unit (NTU). While suspended particles could be deleterious to aquaculture enterprises (Timmons and Ebeling, 2002), the presence of microalgae in rearing tanks (green water) has increased survival, growth, and food conversion index for more than 40 species of fish larvae (Muller-Feuga 2003). However, the reasons for that are not fully understood because phytoplankton cultures are complex mixtures of suspended (live or inert) and soluble organic and mineral substances. Among the main hypothesis to explain the improvements of fish larvae culture in green water is the enhancement in the prey contrast, which would facilitate predation. Since some successful rearing of *Octopus* paralarvae were undertook under "green water" conditions (De Wolf et al. 2011; Dan et al. 2018), we suppose that the hypothesis that water turbidity enhances predation should be better evaluated.

Therefore, we infer that since *O. vulgaris* Type II paralarvae is a visual predator and require live preys, manipulating environmental conditions (i.e., artificial polarized light and turbidity) to favor predation by paralarvae could ultimately improve their feeding rates. By improving feeding rates while also providing adequate natural preys to paralarvae, we could boost their growth and survival rates under laboratory conditions. Thus, the aim of this study is to evaluate if polarized light and water turbidity enhance newly-hatched *Octopus vulgaris* Type II paralarvae feeding rates when fed on copepods (*Acartia lilljeborgi*). In addition, we quantified paralarvae feeding rates on natural prey and

estimate their energy consumption based on the preyed individuals. This is particularly important because we can estimate the daily energy consumed by the paralarvae, allowing comparing results from different studies despite the selected prey, as well as estimate how much of the metabolic rates are supplied by ingestion in the first feeding.

Materials and Methods

Ethics statement

The authors hold an authorization (number 61506-1) from the Brazilian Ministry of the Environment (MMA) to collect, transport and maintain *Octopus vulgaris* for scientific purposes. Despite the absence of laws and regulations regarding invertebrates in Brazil, we have followed the European guidelines for the care and welfare of Cephalopods in research (Fiorito et al. 2015).

Broodstock and paralarvae maintenance

Broodstock was obtained by divers at Bombinhas Beach (27°08'36"S 48°28'43"W) in Santa Catarina State, Brazil, and transferred to the Cephalopod Early-Life Stages Laboratory (www.cephalopod-early-life.com) at the Center for Marine Studies, University of Paraná, Brazil. In the laboratory, they were acclimatized to a recirculation system composed of three 310-500 L cylindrical tanks, an activated carbon filter, a 230 L biofilter, and an 18 W UV lamp. The tanks were cleaned and siphoned daily to remove excreta, food residues, and other organic suspended particles. Seawater was used to replace the disposed water, totaling up to 40% of the total volume per day. Live and thawed crabs (*Callinectes sapidus* and *Callinectes danae*) were used to feed the broodstock until apparent satiety. Ammonium, nitrite, and nitrate were measured every other day and were within the optimal range for the species (Vidal et al. 2014). The temperature was maintained at 22ºC ±1.5 ºC; salinity at 34, pH at 7.8-8.2, and photoperiod was natural. Copulation was observed on the same day males and females were placed in the same tank at a sex ratio of 1:1. Spawning took place 35 days after copulation was first observed and lasted for 26 days. After hatching, paralarvae were transferred to another rearing tank for up to 36 h after hatching until the beginning of the experiments. Paralarvae were maintained in 250 L cylindrical tanks with black walls and white bottom.

Acartia lilljeborgi copepods collection and maintenance

Copepods were collected by plankton tows in Mirassol beach (25°41'10"S 48°27'22"W), Pontal do Paraná, Brazil, using a cylindrical-conical net (300 μm mesh). After transportation to the laboratory, adult *Acartia lilljeborgi* were identified and selected under a dissecting microscope with Pasteur pipettes. Sorted organisms were maintained on 10 L seawater containers with constant aeration, at 34 salinity, 22 ºC temperature, and fed *ad libitum* twice a day with *Chaetoceros müelleri*.

Experimental design

The experimental units were opaque black containers (9 cm diameter, 13 cm height) with a volume of 0.5 L, which was filled with seawater from the paralarvae rearing system. Aeration was supplied by a 3 mm diameter tube directed close to the water surface (horizontal inlet), promoting a gentle water movement without creating air bubbles. Five replicate units were used for each treatment. There were three treatments for polarized light and four for water turbidity (for details see Figures 1 and 2). Three Control replicates were used only with prey (80 L^{-1}) to evaluate possible mortality and methodological errors in the maintenance and prey quantification.

Each replicate unit contained forty copepods carefully counted. Copepods were taken from the culture tanks and counted using a pipette under a dissecting microscope equipped with an ocular micrometer. Paralarvae were randomly collected from the rearing tanks with a small glass container and gently transferred to the experimental units at a proportion of one paralarvae per replicate. Paralarvae are able to capture prey as soon as they hatch, but we have used in the experiments paralarvae between 24 and 36 h after hatching, because at this age their yolk reserve had been partially consumed and more space in the mantle cavity is available for prey digestion (Vidal et al. 2002a; Villanueva and Norman 2008). The experiment lasted 24 h and as soon as the paralarva was placed into the experimental units, the chronometer was started for that particular replica.

The experimental units were checked every 8 h to verify if paralarvae were swimming and alive. After 24 h the experimental units were filtered through a six steps protocol (Figure 3) to collect the remaining copepods. First, the experimental unit content was filtered through a 300 μm mesh and carefully washed three times with seawater from a Pisseti. The content retained over the mesh was displaced into a petri dish, from where the paralarva was collected by a pipette and transferred to another Petri dish. Each paralarva was evaluated by its coloration, behavior and presence/absence of gut contents and measured (mantle length – ML) at the very moment, before anesthesia with 2% magnesium chloride (Messenger et al. 1985) and fixation in 4% buffered seawater formaldehyde

(Vidal et al. 2010). Paralarvae survival rates among each treatment were calculate in percentages. The copepods were fixed with 3 drops of buffered 4% seawater formaldehyde. Later, these copepods were counted to determine the paralarvae daily feeding rates (FR).

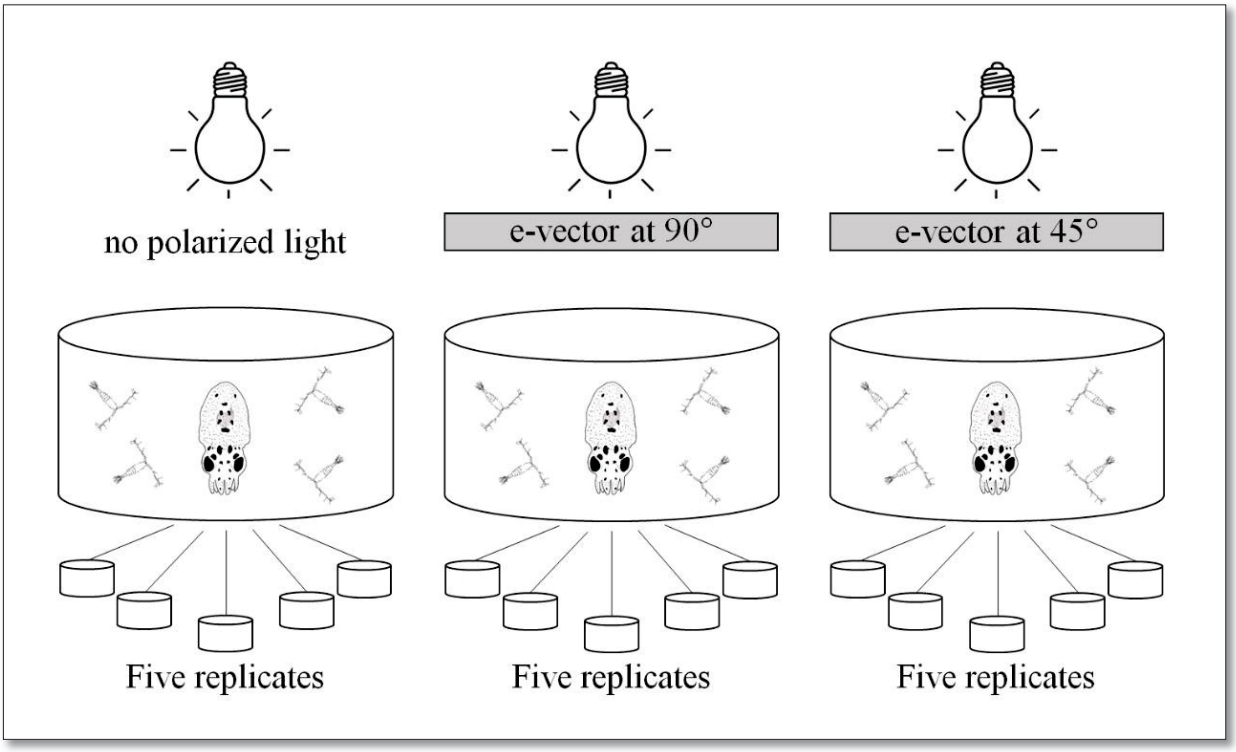

Figure 1 – Schematic design of the experiment conducted to evaluate the influence of polarized light on *Octopus vulgaris* Type II paralarvae feeding rates. Over each treatment there were two LED light bulb source providing 640 lx on the water surface. Treatments with e-vector at 90º and 45º had a PACO® polarizer filter positioned between them and the light source. These filters are represented by the rectangles around the Treatment names. Fifteen replicates with 40 copepods and 1 paralarva each were used in this experiment, which lasted 24 h.

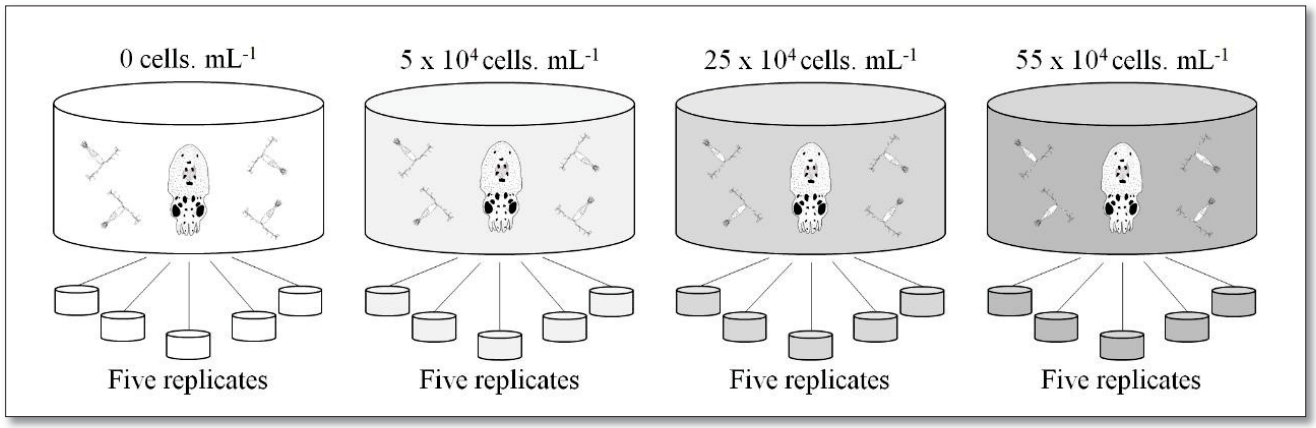

Figure 2 - Schematic design of the experiment conducted to evaluate the influence of water turbidity on *Octopus vulgaris* Type II paralarvae feeding rates. Over each treatment there were two LED light bulb source providing 640 lx on the water surface. Twenty replicates with 40 copepods and one paralarva each were used in this experiment, which lasted 24 h.

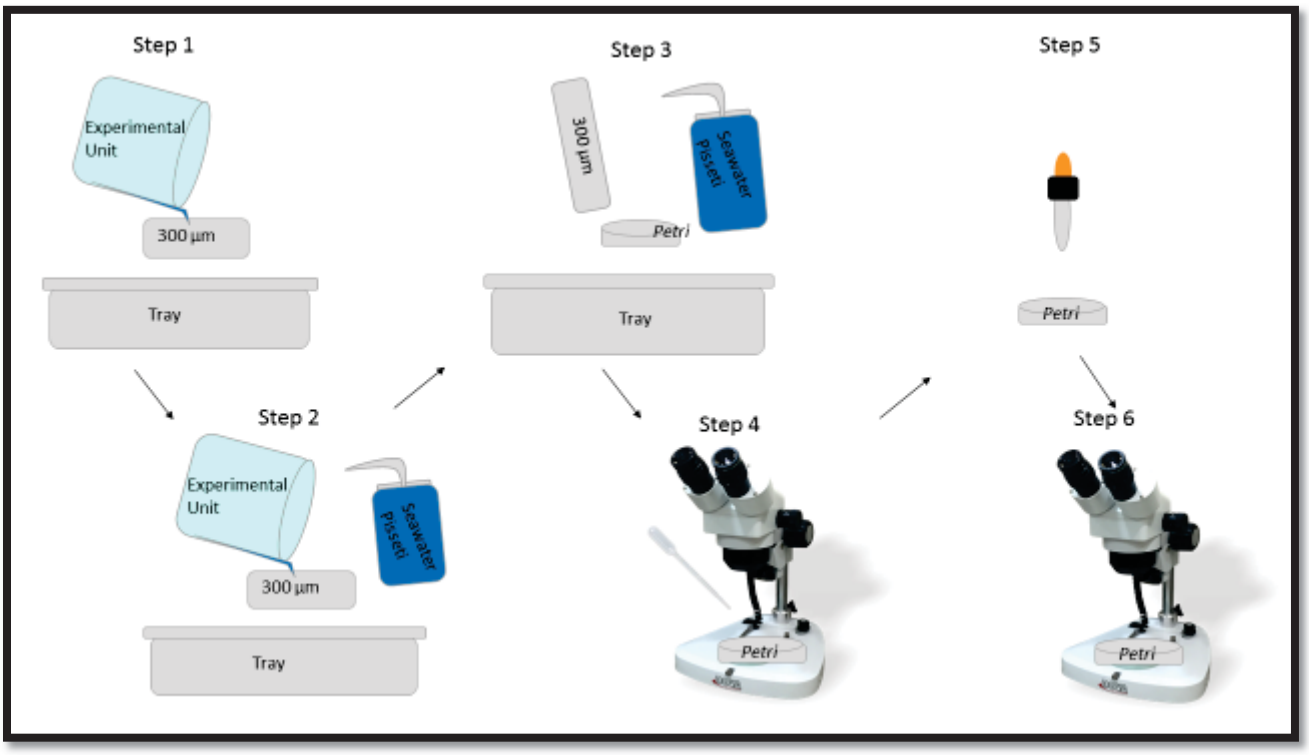

Figure 3 – Diagram of the experimental procedure used to quantify copepods at the end of experiment. The content of each experimental unit was filtered through a 300 µm mesh (step 1) and subsequently three-fold washed (Step 2) to prevent the loss of any copepod on the experimental unit surface. The retained content was displaced into a petri dish, while the mesh was triple rinsed (Step 3). The paralarva was collected by a pipette and transferred to another petri dish to be evaluated and measure (Step 4) under a dissecting microscope. Copepods were fixed with 3 drops of 4% buffered seawater formaldehyde (Step 5) before being quantified under a dissecting microscope (step 6).

Polarized light experiment

Three distinct light treatments were tested: a) no artificially polarized light, b) polarized light with e-vector at 90º, c) polarized light with e-vector at 45º (Figure 2). There were two LED white light positioned over each treatment. Treatments b and c were set up with a PACO polarizing filter between the light source and the experimental units. These filters were placed at 90º (b) and 45º (c) angles. Since the polarizing filters reduce light intensity (Cameron and Pugh 1991), the light sources were maintained at different distances from the experimental units, in order that all of the experimental units received light intensity of 640 lx. Therefore, the light sources were 55 cm above the experimental units at treatment (a), 25 cm above for the treatment (b), and 27 cm above the treatment (c). Light intensity over every experimental unit was measured with an Ld-505 Icel turbidimeter before the beginning of the experiment. The experiment happened in a dark room with an 18h (L): 6h (D) photoperiod and lasted for 24 h. The water temperature was 22.8ºC ± 0.7ºC, and salinity 35.

Turbidity experiment

Aiming to analyze the influence of water turbidity over the paralarvae feeding rates, four treatments with distinct densities of *Isochrysis galbana* were tested (0, 5, 25, and 55 x 10^4 cells.mL^{-1}) (Figure 3). These treatments turbidity were measured with an digital turbidimeter DLA-2500 and were, respectively, 0.09, 3.80, 19.44, and 45.82 NTU (nephelometric turbidity unit). *Isochrysis galbana* was chosen because it is commonly used in paralarvae and fish larvae rearing (De Wolf et al. 2011; Nass et al., 1996, respectively). The selected densities were chosen based on several rearing studies with marine fish larvae (Nass et al. 1996; Planas and Cunha, 1999;Cobcroft et al. 2001;Carton, 2005;) and *Octopus vulgaris* paralarvae (Garrido et al. 2017; De Wolf et al. 2011). The treatments were randomly placed into three thermal boxes to maintain temperature stable. There weretwo LED light tubes (55 cm above the experimental units) over each thermal box to offer a light intensity of 640 lx. The experiment lasted 24 h and the photoperiod was 18h (L): 6h (D). Water temperature was 23.1ºC ± 0.3ºC and salinity 35. Algae concentrations were obtained by dilution of a denser culture with seawater from the paralarvae system. Cell counts were made by direct microscopic with Neubauer chambers.

Observed feeding rates

Daily feeding rates (FR) were evaluated after the subtraction of the number of intact copepods fixed after the end of the experiment from the total number of copepods placed into each experimental unit (n=40). A general FR was established by the mean value of ingested copepods at every Control treatment, as well as from non-significant different treatment. Results are presented as means ± standard deviation (SD) and express the number of copepods ingested per day (copepodsday^{-1}).

Estimated daily energy consumption and metabolic rates

Aiming to extend the application of the quantified FR, it is paramount to transform this information in energy consumption and how much does this energy represent into the paralarvae demand. Therefore, we estimated the Individual Energy Content (IEC, cal.ind^{-1}) of prey based on their dry weight (DW, µg) and energy per mass (EPM, cal. g^{-1}), and the Metabolic Rate (MR, cal.day^{-1}) of paralarvae.

According to Ara (2001), there is a linear correlation between *A. lilljeborgi* prosome length (PL; mm) and its DW (µg): $DW = 6.177\ 10^{-9} PL^{3.029}$. To obtain the dry weight of the prey, the

prosome of all copepods from the Control Treatment (no polarized light and no algae) were measured under a dissecting microscope equipped with an ocular micrometer. Measurement disregarded the pair of large spines on the posterior part of the prosome, since these appendages are fragile and vary in length (Grossmann and Lindsay 2014; Ara, 2001). Mean PL was 1.02 ± 0.05 mm (n = 240). Therefore, the copepods used in the experiment had an estimated DW of 6.62 ± 0.01 μg.

Acartia lilljeborgi energy per mass EPM is very similar to that of *Acartia tonsa* (Ara 2001), in which every 1 g contains 5251 calories (Laurence 1976). Thus, using this information we estimated the *A. lilljeborgi* IEC as 0.035 calories. In order to provide a comparison with other prey of paralarvae found in the literature, we also estimated *Tisbe* sp. and *Artemia* nauplii and metanauplii IEC. Unfortunately, we could not estimatethe IEC of *Palaemon serrifer* zoeae due to lack of information on DW (Itami et al. 1963) and EPM for the zoeae stages. However, adult *P. serrifer* has an estimated EPM between 4.2 and 4.5 cal. DW mg^{-1}(Cummins and Wuycheck 1971).

Artemia nauplii and metanauplii sizes and DW vary according to its cyst diameter (Dhont and Stappen 2003), which varies according to environmental and/or processing factors (Vanhaecket et al. 1983). Therefore, cysts origin influences the *Artemia* nauplii energy content (Stappen 1996). Both Garrido et al. (2017) and Márquez et al. (2007a) have used cysts by INVE AQUACULTURE, Belgium. Based on this company website, most commercialized cysts come from the Great Salt Lake, USA. Therefore, according to Stappen (1996), *Artemia* nauplii mean DW is 2.42 μg. Léger et al. (1987) determine an average EPM of 6540 cal. g^{-1} for *Artemia* nauplii, leading us to estimate its IEC as 0.016 calories. *Artemia* metanauplii DW is 2.31 μg, with an IEC of 0.010 (García-Ortega et al. 1998).

For a *Tisbe* sp. copepod, the DW ranges from 1.1 to 1.9 μg at 27 and 18 ºC, respectively (Parise 1975). Garrido et al. (2017) experiments were conducted at 20 ºC. Consequently, we assumed a DW of 1.9 μg and an EPM content of 3245 cal.g^{-1}, according to Parise (1975). Thus, every ingested *Tisbe* sp. would contain 0.006 calories.

The estimated IEC of the preys were then multiplied by the FR observed to generate values for Daily Energy Consumption (DEC, cal. paralarva^{-1}day^{-1}) of paralarvae. Paralarvae DEC was compared to its MR. Parra et al. (2000) estimated the MR for unfed *Octopus vulgaris* paralarvae as 4.32 nmol O_2 μg^{-1}day^{-1}. At aerobic metabolisms – predominant for cephalopods (O'Dor and Wells 1987) – the energy of each mol of oxygen is 107.1 Kcal (Nelson 2013). Therefore, to convert the MR from oxygen consumption to calories, we used the following reckoning: $MR = t\ OC\ 10^{-9}\ DW\ 1.071\ 10^{5}$. Where t is the time in hours and OC is the oxygen consumption (nmol O_2 day^{-1}paralarvae^{-1}).

An MR for fasting and active paralarvae, like the one that we used, can be estimated by the linear regression obtained by O'Dor and Wells (1987):

$$MR = 0.0043DW^{0.96}1.187^{T},$$

in which MR is expressed in cal.day^{-1}, DW is used as mg and T refers to temperature. This equation was estimated for squids (*Illex illecebrosus*, *Doryteuthis opalescens,* and *Doryteuthis pealeii*) from all life stages and fed *ad libitum*. Despite being obtained for squids, we considered, this equation more appropriate for *Octopus* paralarvae, which are planktonic, than the equation obtained for adults *Octopus vulgaris* (Wells et al. 1983). That is because animals with a DW under 40 g would not be well represented since paralarvae are active and swim upwards (O'Dor and Wells 1987).

Paralarvae DW (0.290 ± 0.016 mg) was obtained from 20 animals randomly selected from the rearing tanks. Four crucibles with 5 paralarvae each were dried at 60 ºC for 24 h before being cooled at room temperature and measured at an Ohaus balance, with a precision of 0.00001 g.

Statistical and data analysis

Lavene test (1960)and Shapiro-Wilk test (1965)were performed to verify, respectively, equality of variances and data normality at 5%. Parametric data were evaluated by a One-way ANOVA correlating polarized light to feeding rates (Zar 1999). Non-parametric data were evaluated by a Kruskal test correlating water turbidity to feeding rates. All data analysis was performed on RStudio software.

Results

Polarized light experiment

Newly-hatched paralarvae have shown a feeding rate of 3.8 ± 2.28, 5.0± 3.16, and 5.4 ± 1.82 copepods day^{-1} when exposed to regular light, and polarized light with the e-vector at 90º and at 45º, respectively (Figure4). Individual variability was high with minimal and maximum observed FR as 1 and 10 copepods day^{-1}, respectively. The results were equality and normally distributed but no significant difference was found (p-value = 0.562).

Survival rates were 100% on both treatments with artificially polarized light and 80% on the regular light treatment. Only one paralarva from the regular light treatment was found dead at the end of the experiment. This particular paralarva have eaten three copepods and had full gut content at the

end of the experiment. The death of the paralarva probably happened after 20+ h of experiment, since lack of movements was observed 2 h before the end of the experiment.

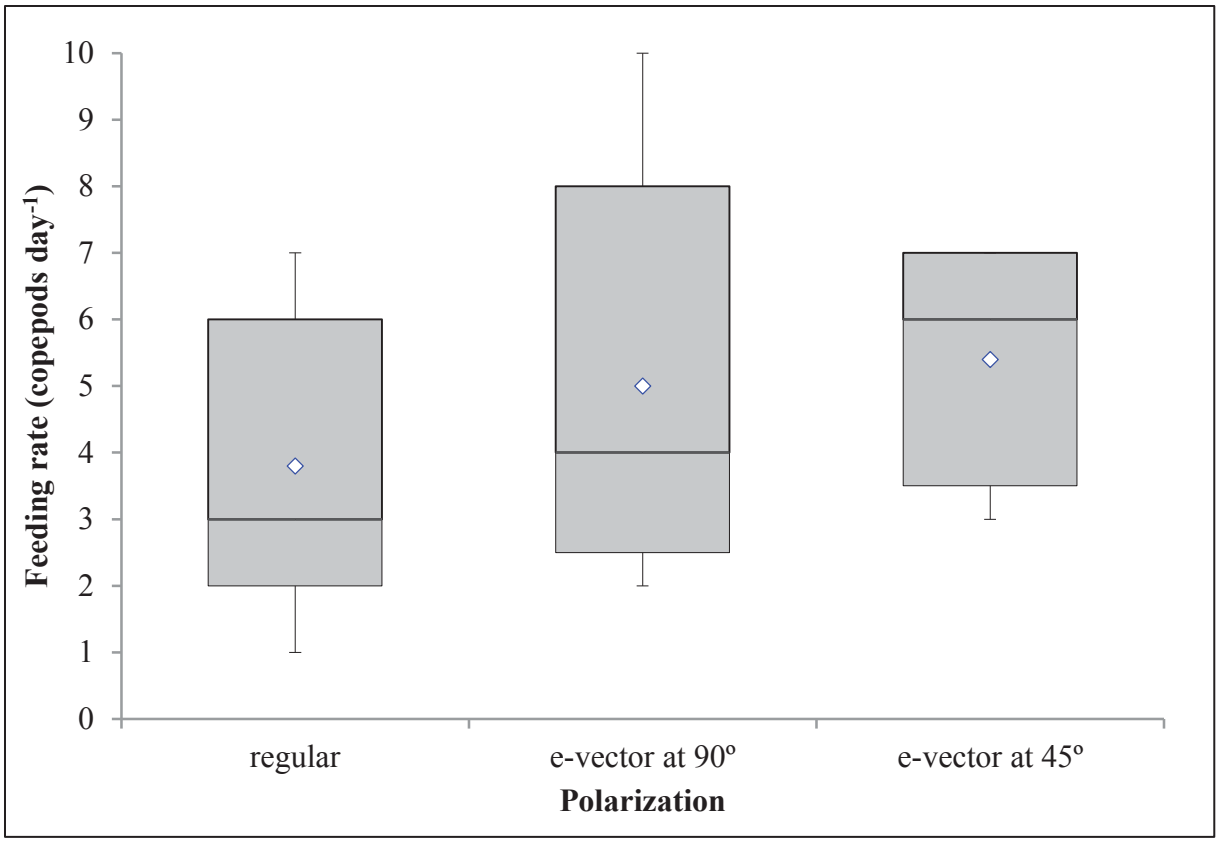

Figure 4 – Boxplot representing the feeding rates of newly-hatched *Octopus vulgaris* Type II paralarvae when fed on *Acartia lilljeborgi* at a density of 80 copepods.L^{-1} at three distinct light treatments. Each horizontal line of the rectangle, bottom-top, represents one quartile (1st, 2nd, and 3rd respectively). Minimum and maximum values beyond the quartiles are represented, respectively, by the trace under and over the rectangle. Mean values are represented by the blue diamond. No significant difference was found (p-value = 0.562).

Water turbidity experiment

Newly-hatched paralarvae have shown a feeding rate of 3.4 ± 2.07, 4.4 ± 1.34, 3.6 ± 2.07, and 1.4 ± 1.34copepodsday^{-1}on the "green water" environment at 0, 5, 25, and 55 x10^4 cellmL^{-1}, respectively (Figure5). Minimal and maximum individual observed FR was 0 and 7 copepods day^{-1}, respectively. The results were non-parametric and no significant difference was found (p-value = 0.428).

No paralarval mortality was registered during the experiment. However, all paralarvae from the higher algae density were found immobile at the bottom of the experimental units at the end of the experiment. Regardless of that, they have shown healthy behavior on the *Petri* dishes, being able to swim and even attacking prey just after being taken out of the experimental unit at the end of the

experiment. The observed attack was made by the paralarva that has not eaten during the experiment. Coloration and behavior were similar to the Control paralarvae.

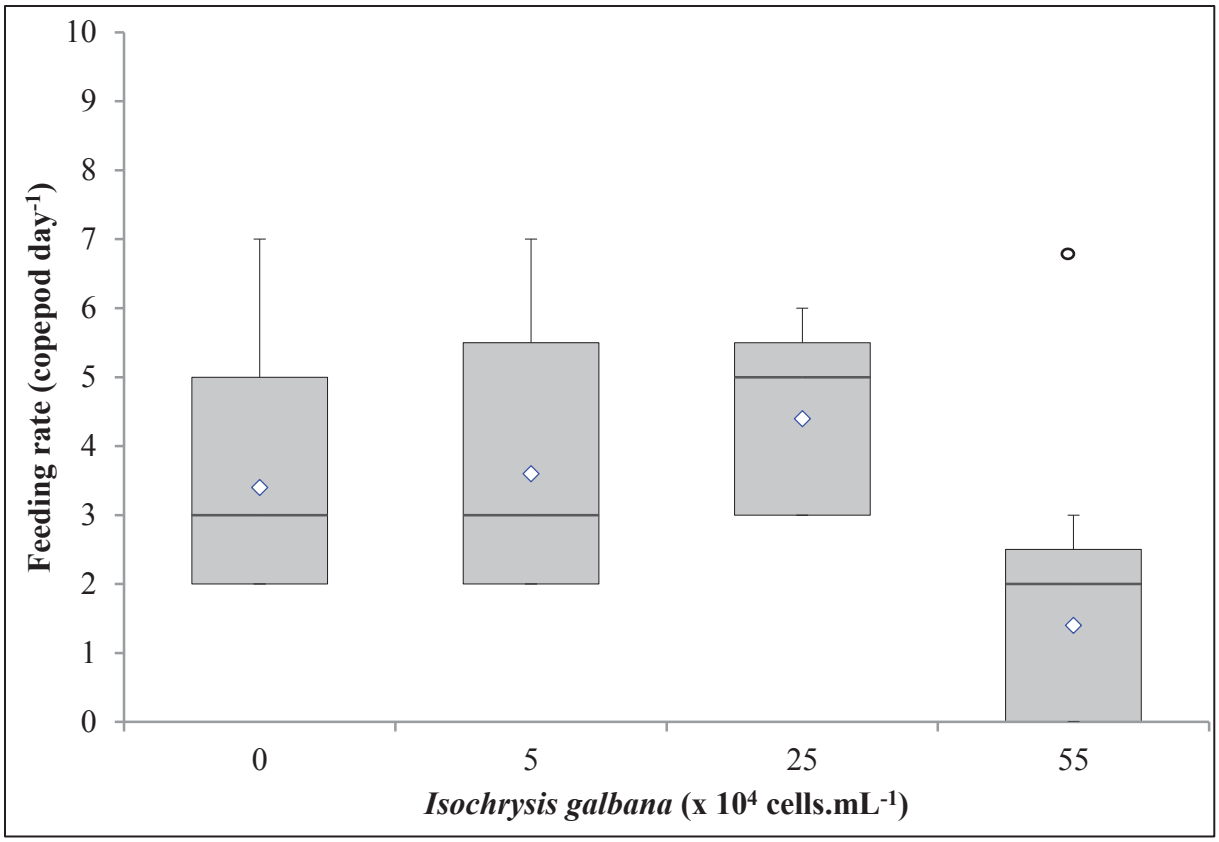

Figure 5 – Boxplot representing the feeding rate obtained for newly-hatched *Octopus vulgaris* Type II paralarvae when fed on *Acartia lilljeborgi* at a density of 80 ind.L^{-1} at four distinct algae concentration. Each horizontal line of the rectangle, bottom-top, represents one quartile (1st, 2nd, and 3rd respectively). Minimum and maximum values beyond the quartiles are represented, respectively, by the trace under and over the rectangle. Mean values are represented by the blue diamond. Outlier (value 150% bigger than the difference from 1st to 3rd quartiles) is displayed as a black circle. No significance was found with p-value at 0.482.

Observed feeding rates, estimated energy consumption, and estimated metabolic rates

Since no significant difference was found on the feeding rates between treatments, we considered all treatments to obtain our mean FR, which was 3.86 ± 2.26 copepods day^{-1}. Multiplying the FR mean value by the estimated IEC of *Acartia lilljeborgi* gave us an average DEC of 0.135 cal. day^{-1}. The same calculation was made for the observed FR of previous studies (Itami et al. 1963; Márquez et al. 2007a; Garrido et al. 2017) and results can be seen in Table 1.

Table 1–Observed feeding rates and estimated daily energy consumption of *Octopus vulgaris* paralarvae.

	Prey					Paralarvae			Experimental conditions			Source
	Species	ρ (ind.L^{-1})	L (mm)	DW (µg)	IEC (cal.ind^{-1})	FR (preyparalarva^{-1} day^{-1})	DEC (cal.paralarva^{-1} day^{-1})	DW (µg)	V (mL)	T (ºC)	LI (lx)	
***O. vulgaris* Type II**	*Acartia lilljeborgi*	80	1.02 ± 0.05	6.62 ± 0.01[b]	0.035[f]	3.9 ± 2.3	0.135	240 ± 16	500	23	640	Present study
***O. vulgaris* ss**	*Artemia* nauplii	1000	0.49[a]	2.42[c]	0.016[g]	31.0 ± 3.6	0.491	n.a	50	20	100	Garrido et al. 2017
***O. vulgaris* ss**	*Tisbe* sp.	1000	n.a.	1.9[d]	0.006[h]	15.4 ± 3.0	0.095	n.a	50	20	100	Garrido et al. 2017
***O. vulgaris* ss**	*Artemia* metanauplii	9400	0.85	2.31[e]	0.010[i]	0.8 - 16.0	0.008- 0.160	n.a	9.2	22.6	750	Márquez et al. 2007a
O. sinensis	*Palaemon serrifer*	n.a.	n.a.	n.a.	n.a.	3.0 - 5.0	-	n.a	n.a.	25	n.a.	Itami et al. 1963

Notes: ρ = density; L = length; DW = dry weight; IEC = prey individual energy content; FR = feeding rate; DEC = Daily energy consumption; V = volume; T = water temperature; LI = light intensity; n.a. = not available on the study; a) Dhont and Stappen 2003; b) value estimated by linear regression (Ara 2001); c) value suggested by Stappen (1996); d) value suggested by Parise (1975); e) values obtained from García-Ortega et al. 1998; f) value calculated based on Laurence (1976); g) value calculated based on Léger et al. (1987); h) value calculated based on Parise (1975); i) value calculated based on the macromolecular composition by García-Ortega et al. 1998.

Octopus vulgaris paralarvae MR was estimated at 0.136 and 0.743 cal. day^{-1} when calculated based on Parra et al. (2000) and O'Dor and Wells (1987), respectively. Therefore, estimated DEC represents 99% of the MR of a fasting paralarvae on 1 mL of water (Parra et al. 2000) and only 18% of the MR for squids from all life stages and fed *ad libitum* (Table 2).

Table 2–Estimated metabolic rates and their correlation to Daily Energy Consumption for *Octopus vulgaris* Typo II paralarvae.

DEC (cal. paralarva^{-1}day^{-1})	**MR (cal. paralarva^{-1}day^{-1})**	**DEC.MR^{-1} (%)**	**Source**
0.135	0.136	99	Parra et al. 2000
	0.743	18	O'Dor and Wells 1987

Notes: DEC: Daily Energy Consumption was estimated by multiplying the individual energy content of *Arcartia lilljeborgi* (Laurence 1976, Ara 2001) by the observed feeding rate. MR: Metabolic rate was estimated either on the measured value (Parra et al. 2000) as by the linear regression (O'Dor and Wells 1987) $MR = 0.0043DW^{0.96}1.187^{T}$, in which MR is expressed in cal.day^{-1} and DW is used as mg.DEC.MR^{-1}: the proportion of DEC per MR calculated either by Parra et al. 2000 or O'Dor and Wells 1987.

Discussion

These results have shown that there is no correlation between feeding rate and light polarization or water turbidity for newly-hatched *Octopus vulgaris* Type II paralarvae. Nevertheless, this is the first study to quantify *O.* cf. *vulgaris* feeding rates using natural prey (planktonic copepods) and to estimate the corresponding energy consumption. This information can offer substantial insights for better understanding the trophic ecology and energy budget of *Octopus* paralarvae in the wild, as well as their daily nutritional needs under cultivation.

The results regarding the polarization light experiment are in accordance with Garrido et al. (2017) and Martín (2017), which have found no significant differences between polarized and unpolarized light on the feeding rates of *O. vulgaris ss* paralarvae. One possible explanation for the lack of interference of polarized light on FR was suggested by Martín (2017) who mentioned that polarization vision probably improves with age when paralarvae inhabit deeper waters (100-200 m) (Roura et al 2019). Similarly, Cartron et al. (2013) suggest that vision in cephalopods is developed through experience, since only 20% of newly-hatched cuttlefish tested showed polarization sensibility, against 100% of the 30 day-old ones. Other studies suggest that cephalopods vision is an experience-based learning process and stimuli can play an important role. Imarazene et al. (2017) have shown that cuttlefish embryos can discriminate objects out of the egg and Darmaillacq et al. (2008, 2017) have

demonstrated that cuttlefish prenatal experiences play a role in their postnatal behavior, changing prey preferences. It is important to note, however, that cuttlefish hatchlings are considerably larger and more developed than *O. vulgaris* paralarvae (Nixon and Mangold 1998). Nevertheless, all cephalopods have direct development and *Octopus* paralarvae hatch out as a visual predator (Boletzky and Villanueva 2014).

If vision is experience-based, the environment under laboratory conditions could hinder polarized light vision development. The lack of predator stimuli and low prey diversity and variability can be accounted for this. Feeding rate studies taking older paralarvae are essential to cast some light on these questions. More important, though, are studies that provide evidence of polarized vision in *Octopus vulgaris* paralarvae. So far, there are no studies demonstrating that *Octopus* vulgaris vision is polarized. We could only suppose that from previous studies with squid paralarvae (Shashar et al. 1998) and adult cephalopods (Shashar and Cronin 1996 – octopus; Shashar and Hanlon 1996 – squids; Shashar et al. 2000 – cuttlefish).

Another reason for the lack of correlation between polarized light and feeding rates could be the fact that increasing predator's perception of the prey does not affect predation success. For *Octopus vulgaris* and other cephalopod paralarvae (Hernández-Garcia et al. 2000, Messenger 1968), the predation process is divided into three stages: attention, positioning, and attack. Increasing the perception distance (attention) does not result, necessarily, in an increment in the attack success. Shashar et al. (1998) demonstrated that under polarized light the attack distance of squid hatchlings was 70% greater than under depolarized light. However, attack success rates were statistically similar ($P>0.05$) at 86% (polarized light) and 74% (depolarized light).

The absence of correlation between one of the stages and the whole of the predatory process was also observed when the influence of water turbulence on fish predation was investigated. Mackenzie et al. (1994) have shown that higher predator-prey encounter rates do not result, necessarily, in greater feeding rates. These authors emphasized that what hinders predation success is that turbulence reduces the encounter time, preventing fish larvae from positioning themselves and attacking the prey.

Under polarized light, what could reduce and impact the attack success is the increase in the predator-prey distance. Maximum reaction distance tends to be two to four-fold of *O. vulgaris* paralarvae length (Villanueva et al. 1996). This maximum reaction distance is influenced by the predator capability to identify its prey as well as by the predator and prey swimming abilities. When attacking prey, *Octopus vulgaris* paralarvae display forward swimming in order to keep visual contact with the prey (Villanueva et al. 1996). However, this swimming behavior is slower than the regular backward swimming for two main reasons. First, the turn created in the funnel for forwarding

displacement lowers jet efficiency. Second, the forward movement increases hydrodynamic drag (Villanueva et al. 1996). Ergo, if polarized light enhances prey detection, the attack success could be diminished by the great distance and reduced swimming speed.

Swim speed might also have been impacted by the water turbidity due to the increase in seawater viscosity. It has been acknowledged that increasing algae concentration heighten water viscosity (Petkov and Bratkova 1996; Adesanya et al. 2012), which has been proven to impact swimming speed on fish larvae that operates at low and intermediate Reynolds number (Fuiman and Batty 1997). It is likely that *O. vulgaris* paralarvae operate at similar bands of Reynolds number since squid hatchlings of the same size have been proven to (Bartol et al. 2008; Martins et al. 2010). Therefore, water viscosity has probably played an important role in paralarvae swimming abilities, hampering their displacement, thus hindering predation (Mackenzie et al. 1994). This could explain the low feeding rates observed on the higher cell concentrations treatment (55 x 10^4 cells.mL^{-1}). First, because all paralarvae from this treatment were immobile at the bottom of the experimental unit at the end of the experiment but started swimming as soon as the experimental units were rinsed and paralarvae and copepods were displaced in the *Petri* dishes. Second, even the unfed paralarvae (FR = 0) had coloration similar to control and were trying to prey on copepods as soon as they were transferred to the *Petri* dish at the end of the experiment. Third, notwithstanding the maximum FR (3 copepods day^{-1}) on this treatment is far from the others (Figure 5).

The boxplot analyzes (Figure 5) can bring some more noteworthy perceptions. It is interesting to note that the 25 x 10^4 cell.mL^{-1} treatment has demonstrated the highest values among the replicates that consumed less copepods (3 copepods day^{-1}), among the mean values (4.4 copepods day^{-1}), and among the median values (5 copepods day^{-1}). Thus, despite no significant differences, this Treatment has shown that some slighted higher algae density could perhaps offer even better outcomes. Therefore, it would be important to investigate intermediate algae concentrations (between 25 and 55 x 10^4 cells.mL^{-1}) to ensure that water turbidity does not enhance FR.

Future FR studies that amplify the sample size is also essential. It is interesting to note about the polarized light results (Figure 4) that the highest individual FR and the highest median FR were both obtained under polarized light: 10copepods day^{-1} under the e-vector at 90º and 6 copepods day^{-1} under the e-vector at 45º. The lack of significant differences either for turbidity or for polarized light could be due to the small sample size, which enhances individual variability. Individual variability with potential impact on survival rate is a known fact in fish (Moran 2007), crustaceans (Barros and Valenti 1997) and cephalopods (Vidal et al. 2002a, 2006; Márquez et al. 2007b) and could also influence feeding rate in paralarvae (Vidal et al. 2006). Paralarvae hatch out with high variability in yolk reserves (Vidal et al. 2002b). Therefore, it would be expected that paralarvae with larger yolk

content at the beginning of the experiment might reduce prey searching (Villanueva et al. 1996)and feeding rate, at least partially explaining the within-treatment variability.

Indeed, individual variability seems important to be evaluated when comparing DEC to MR (Table 2). Considering the MR established by Parra et al. (2000) for fasting paralarvae on small water volume (1 mL), the DEC estimated based on our observed FR represents almost the total amount of energy consumed by the paralarvae. This would suggest that 4 copepods day^{-1} would be enough for feeding a paralarva during the first days of life. However, the FR established by the present study used paralarvae that were swimming and preying. This suggests that the MR produced by the model of O'Dor and Wells (1987) would be adequate for these paralarvae metabolism as it was obtained for active (swimming and feeding) squids. Nevertheless, by doing so, the estimated DEC (0.135 cal. $paralarva^{-1}$ day^{-1}) would represent only 18% of the MR of paralarvae. This would imply that either the model overestimated the MR of paralarvae or more than 80% of the energy necessary to maintain the metabolic rate of newly-hatched paralarvae would come from their yolk reserve.

In order to provide a more comprehensive view of paralarvae daily feeding rates and energy requirements, we compiled previous information on *O. vulgaris* FR(Table 1). Itami et al. (1963) obtained a feeding rate of 3-5 zoeae of *Palaemon serrifer* $paralarvae^{-1}$ day^{-1} for *Octopus sinensis* newly-hatched paralarvae (3-5 mm), at 25 ºC. On the other hand, Márquez et al. (2007a) obtained a feeding rate of 0.8-16 *Artemia* metanauplii day^{-1} for *O. vulgaris* paralarvae when using experimental units of only 9.2 mL, prey density of 9.4 metanauplii mL^{-1} and light intensity of 750 lx. Garrido et al. (2017), working with the same species found much larger rates either for *Artemia* (31±3.6 *Artemia* nauplii day^{-1}) as for *Tisbe* sp. (15,4±3.0 copepods day^{-1}), using 50 mL glass beaker as experimental units with 1 *Artemia*/copepod mL^{-1} and under 100 lx light intensity (Table 1).

Considering that different experiments tested different species and prey under different conditions, it is not possible to make direct comparisons between the FT of paralarvae. Nonetheless, the estimated DEC (Table 1) should also be taken into account with the FR since it indicates how much energy the paralarvae requires per day. Therefore, while the paralarvae FR measured when *Artemia* nauplii is offered as prey is eight-fold higher (31 *Artemia* day^{-1}) than that found in the present study FR (3.9 copepods day^{-1}), the DEC gap is 55% smaller (0.491 vs 0.135 cal. $paralarva^{-1}$ day^{-1}). One possible explanation for this apparent discrepancy is because *A. lilljeborgi* IEC is more than the double of *Artemia* nauplii. Consequently, a paralarva would need many *Artemia* nauplii to support their daily metabolism. Since it was observed that there is a reduction on *Artemia* DW and IEC through the first hours of life after hatching (Dhont and Stappen 2003), *Artemia* metanauplii mean DEC (0.084 cal. $paralarva^{-1}$ day^{-1}) is the lowest value, just after *Tisbe* sp. (0.095 cal. $paralarva^{-1}$ day^{-1}). *Tisbe* sp. low IEC is related to the fact that it is a small sized benthic species and, therefore, its energy content is

lower than nektobenthic and planktonic species (Company and Sardà 1998). The evaluation of the DEC could be more reliable if future studies would supply paralarvae DW, prey size, prey DW, prey density, and prey IEC.

In addition to the energy content of the prey, it would also be important to take into account the significance of phospholipids and n-3 polyunsaturated fatty acids in paralarvae nutritional requirements (Navarro and Villanueva 2000, 2003; Seixas et al. 2010; Guinot et al. 2013; Garrido et al. 2016). Paralarvae natural prey as mysid, decapod zoeae and marine copepods (Drillet et al. 2006; Roura et al. 2012) are rich in n-3 polyunsaturated fatty acids, with a good amount of docosahexaenoic acid (DHA) (Navarro and Villanueva 2000, 2003). *Artemia*, on the other hand, metabolizes DHA into eicosapentaenoic acid (EPA), showing only traces of it if not enriched(Navarro et al. 1999; Navarro and Villanueva 2000; Reis et al. 2017). Wild calanoid copepods contain almost twice the polar lipid content of enriched *Artemia* (McEvoy et al. 1998). Polar lipids are important because they are readily digested and facilitate other lipids digestion (Dhont and Stappen 2003). Therefore, the different FR of paralarvae found by the different might have been influenced by prey nutritional content.

Besides prey size and energy content, prey density plays a major role in the predation success of *O. vulgaris* paralarvae. Márquez et al. (2007a) have shown that this species functional response is a Type III, which means that prey density is directly proportional to the attack rate (Hassel 1978). In addition, D. O. Ortiz (personal observation) has observed a significant increase in feeding rates with increasing prey density (from 20-80 ind.L^{-1}). This call the need to determine ideal prey densities for paralarvae during larviculture and to establish a rearing feeding protocol. Nevertheless, it isof note that Iglesias et al. (2006) found no significant differences when offered 100, 500 and 1000 *Artemia*L^{-1} for *O. vulgaris* paralarvae. Therefore, there must be a prey density in which FR stabilizes. Finding this value would be very important both to aquaculture entrepreneurship as well as for understanding the feeding ecology of paralarvae. For aquaculture, the excess of prey results in higher expenses and increase the levels of nitrogenous compounds and carbon dioxide on the water while also reducing oxygen availability (Vidal and Boletzky 2014). Within an ecology context, it would help to understand the pressure that newly-hatched *Octopus vulgaris* paralarvae put on their prey at the second trophic level of the marine food web. Since each *O. vulgaris* female produces from 100.000 to 600.000 eggs (Mangold 1983, Iglesias et al. 1997), the biomass demanded to sustain the high metabolism of paralarvae must be considerable.

The prey densities offered to paralarvae in the present study (80 L^{-1}), although relatively lower than that used in other studies (Garrido et al. 2017, Márquez et al. 2007a), seems a reasonable value to be considered either for FR experiments and for paralarvae rearing. Garrido et al. (2017) have suggested that polarized light did not interfere in predation because confined environment already

offers easy conditions for finding prey. High prey densities facilitate, even more, predator-prey encounter (Márquez et al. 2007a). Therefore, we drastically reduced the number of prey offered based on the fact that *Doryteuthis opalescens* paralarvae were successfully reared to the juvenile phase with prey density within the rage of 50 -150 prey L^{-1} (Vidal et al. 2002a).Additionally, the selected prey density was 10-fold of the obtained FR, which suggests that prey density offered was enough.

In addition, the choice of size, shape, and volume of the experimental units used in feeding experiments is another important factor to be considered. The discrepancies between the experimental units used to obtain feeding rates of paralarvae were very large (9.2 mL vs. 50 mL vs 500 mL) among published studies (Table 1). It is well documented that tank size influences reproduction, growth and feeding in fish rearing (Lika et al. 2015; Buchet et al. 2008 Goolish et al. 1992; Boeuf and Gaignon 1989). In this context, the volume 9.2 mL used by Márquez et al. (2007a) to obtain feeding rates of paralarvae seems too small and inadequate, since it might have prevented paralarvae normal swimming and feeding behavior. In fact, even a 50 mL experimental unit seems too small to allow extrapolating results to large rearing tanks, where small-scale turbulence is an inherent part of tank circulation (Timmons and Ebeling 2002). Turbulence has proven to considerably impact fish larvae predation (Rothschild and Osborn 1988, Mackenzie et al. 1994) and requires to be evaluated in future FR studies of *Octopus* paralarvae.

Due to the careful methodology applied in the present study to quantify paralarvae FR using their natural prey (copepods), our results are reliable and important data to understand the daily feeding requirements of *Octopus* paralarvae under culture conditions. This information will have special value in establishing a feeding protocol for rearing paralarvae. Also of importance, the copepod species used as prey in the present study is very abundant and of easy accessibility in nature (Salvador and Bersano 2017) and has the potential to be produced in large-scale (Bersano 2003).The present study has also suggested a protocol for future FR studies and raised questions about polarized light and water turbidity. Such as the following: is the vision of *Octopus vulgaris* paralarvae polarized? Does polarized light or water turbidity amplify the distance in that *O. vulgaris* paralarvae perceives the prey? Does polarized vision development is dependent on stimuli and experience and therefore age?

Conclusion

No correlations were found between polarized light or water turbidity and feeding rates in newly-hatched *Octopus vulgaris* Type II paralarvae. However, it was possible to quantify feeding for these paralarvae when fed on natural prey *Acartia lilljeborgi*, which might be an important reference for establishing of feeding protocols during larviculture. It was also possible to develop a more robust

protocol for feeding rates studies, which might facilitate comparison among different species and preys.

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