

Coelenterazine detection in five myctophid species from the Kerguelen Plateau

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Abstract

Myctophidae comprise the largest biomass of mesopelagic fishes in the Southern Ocean. This family has the ability to emit light – bioluminescence – via specific organs called photophores, and from the head and caudal organs. Through biochemical analyses, this study reveals that coelenterazine is the substrate for light reaction in the five most common myctophid species from the Southern Ocean. The results also support the hypothesis of the acquisition of this molecule via the food chain and reveal the presence of two stabilised storage forms of coelenterazine (enol-sulfate and dehydrocoelenterazine) within various tissues of the studied species. Finally, detection of coelenterazine in the gonads also suggests the potential maternal transfer of the bioluminescence capability to the offspring.

Détection de la coelentérazine chez cinq espèces de Myctophidae du plateau des Iles Kerguelen

Résumé

Les Myctophidae représentent la plus grande biomasse de poissons mésopélagiques de l'océan Austral. Cette famille possède la capacité d'émettre de la lumière – bioluminescence – via des organes spécifiques appelés photophores, ainsi que de la tête et des organes caudaux. A travers des analyses biochimiques, cette étude révèle que la coelentérazine serait le substrat utilisé pour la réaction lumineuse chez cinq espèces de Myctophidae les plus communes de l'océan Austral. Les résultats soutiennent également l'hypothèse de l'acquisition de cette molécule via la chaîne alimentaire ainsi que le stockage de la coelentérazine sous deux formes stabilisées (énol-sulfate et déhydrocoelentérazine) au sein de plusieurs tissus des espèces étudiées. Finalement la présence de coelentérazine dans les gonades suggère également un potentiel transfert maternel des capacités lumineuses à la descendance.

Keywords: Myctophidae, bioluminescence, coelenterazine, dehydrocoelenterazine, enol-sulfate, biochemical assays

Introduction

Coelenterazine is a common substrate for light emission found among bioluminescent organisms, from ctenophores to vertebrates (Morin and Hastings, 1971; Cormier, 1978; Shimomura et al.,

1980; Hastings, 1983; Campbell, 1988; Campbell and Herring, 1990; Mallefet and Shimomura, 1995; Deshmukh, 2017). Its acquisition through the oceanic food web is assumed to be the main mechanism explaining the widespread use of this

compound among various distantly related marine clades (Shimomura et al., 1980; Hastings and Morin, 1991).

Myctophidae are bioluminescent meso/bathypelagic fishes that are widely distributed in the world's oceans (Pakhomov et al., 1996; Turner et al., 2009), and they represent the most abundant and largest vertebrate biomass in the Southern Ocean (Sabourenkov, 1991; Hulley and Duhamel, 2011). Myctophidae species are known to perform diel migration; ranging between 200 to 1 000 m depth during daytime and between 200 m to the surface at night (Gjøsaeter and Kawaguchi, 1980). The enormous biomass represented by this family is a food resource for many Southern Ocean organisms such as penguins, seabirds, seals, whales, fishes and squids (Sabourenkov, 1991; Koz, 1995; Raclot et al., 1998; Phillips et al., 2001; Lea et al., 2002; Cherel et al. 2008; Vacquié-Garcia et al., 2012). Despite the abundance of myctophids, their bioluminescence remains poorly known (Barnes and Case, 1974; Krönström and Mallefet, 2010). Nevertheless, myctophid studies already highlight the role of the light emitted (Case et al., 1977) and some potential bioluminescence control (Anctil and Gruchy, 1970; Anctil, 1972). Moreover, genetic investigations indicate the lack of bacterial symbionts within the light organs (photophores) of these midwater fishes (Haygood et al., 1994). Previous studies mentioned coelenterazine (the luciferin) as the substrate for the production of light for some myctophid species. In addition to the detection within the photophores, huge amounts of this luciferin were also detected in the liver and digestive tract from different species supporting the trophic acquisition hypothesis and eventually the storage within organs under stabilised forms (enol-sulfate, dehydrocoelenterazine, protein-protein bound form) (Inoue et al., 1977; Shimomura et al., 1980; Mallefet and Shimomura, 1995).

In this work, five myctophid species from the Kerguelen Plateau were studied. Since nothing was known on their luminescence capabilities, we detected and assayed the luminous system, i.e. coelenterazine and luciferase-specific activity in photophores and various tissues of these five myctophid species. Our results support the use of coelenterazine as substrate of the light reaction in Myctophidae. Stabilised coelenterazine storage forms were also detected in different tissues; the presence of both free coelenterazine in the

digestive tract and of its stable forms within fish tissues strongly suggests a dietary acquisition of coelenterazine through the trophic web.

Materials and methods

Live specimens of myctophids were caught by midwater trawling in January/February 2014 during the RV *Marion Dufresne II* MD197/MYCTO 3D cruise off the Kerguelen Plateau. A total of 32 night trawls (depth range: from 35 to 600 m) as well as six daylight trawls were conducted off the east of the Kerguelen Plateau (Figure 1).

Photographs of the animals and their bioluminescence were taken with a Canon 1Dx camera (Figure 2). Live spectrum of the light emission was measured on the photophores of three specimens of studied species using a Hamamatsu microspectrophotometer (C10083) equipped with an optic fibre (A976201).

For biochemistry analysis, all specimens were immediately frozen at -80°C on board and transferred under dry ice to the marine biology laboratory in Louvain, where they were stored at -80°C until use. For each specimen, caudal and lateral photophores, luminous caudal organs (infra and/or supra, if present), skin, muscle, stomach, intestine, liver and gonads (if present) were dissected on ice, weighed, then extracted and tested separately for the luciferase activity and the coelenterazine content. To prevent any contaminations, each assay was performed independently which means that between each sample, grinding material was cleaned with methanol or phosphate buffer, rinsed with distilled water then dried. Coelenterazine can be found in various forms: (i) free and unstable, or (ii) stable by being bound to proteins or in enol-sulfate or dehydrocoelenterazine forms (Cormier et al., 1970; Inoue et al., 1976; Takahashi and Isobe, 1994). To extract the free and bound coelenterazine, each sample was ground with 10 volumes of cold methanol following Shimomura (2012), and 5 μl of the extract was mixed with 195 μl of Tris buffer 10 mM, NaCl 0.15 M (pH 7.4). Then, the solution was mixed with 197 μl of Tris-HCl buffer 10 mM, NaCl 0.5 M (pH 7.4) and 3 μl of a solution of *Renilla* luciferase (Prolume Ltd., working dilution of 2 g l $^{-1}$ in a Tris-HCl buffer 10 mM, NaCl 0.5 M, BSA 1%, pH 7.4) in order to trigger the chemical reaction (Renwart and Mallefet, 2013). Under these conditions, 1 ng of pure coelenterazine emitted 2.52×10^{11} photons (Shimomura, 2012).

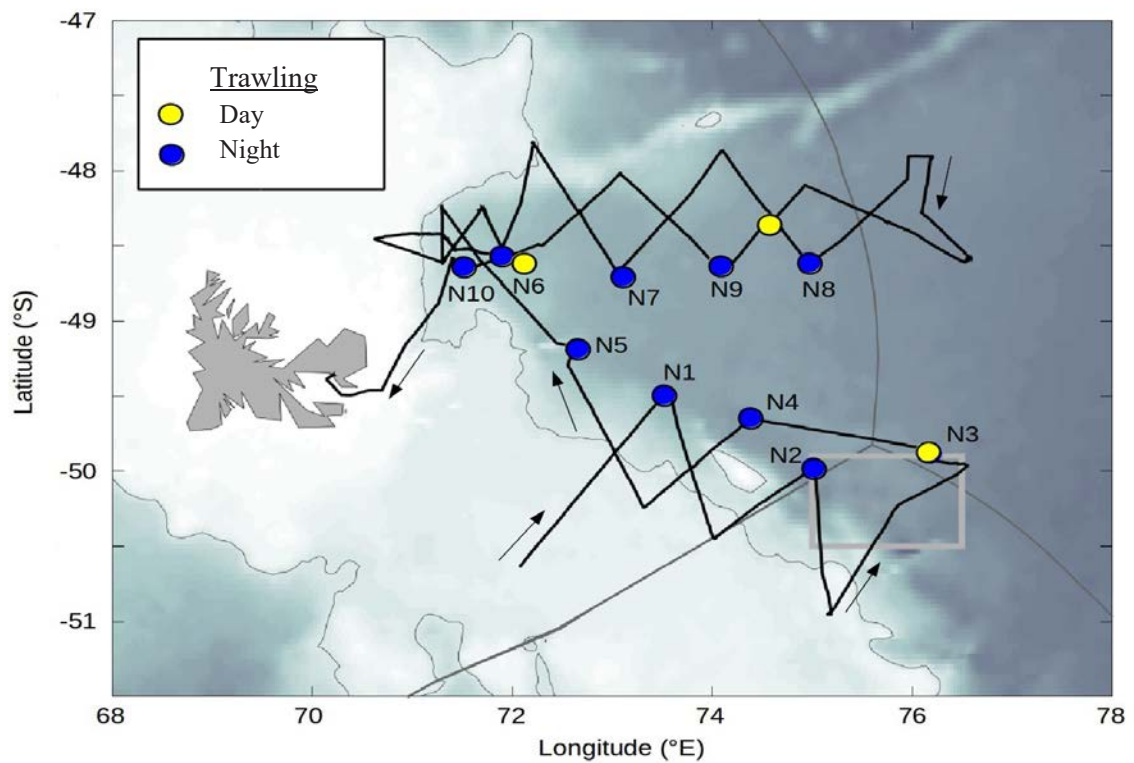


Figure 1: Cruise track of the RV *Marion Dufresne II* MD197/MYCTO (January/February 2014) off the east of the Kerguelen Plateau. Sampling station (N) where multiple trawls were undertaken, mostly by night (blue circle) but also by day (yellow circle).

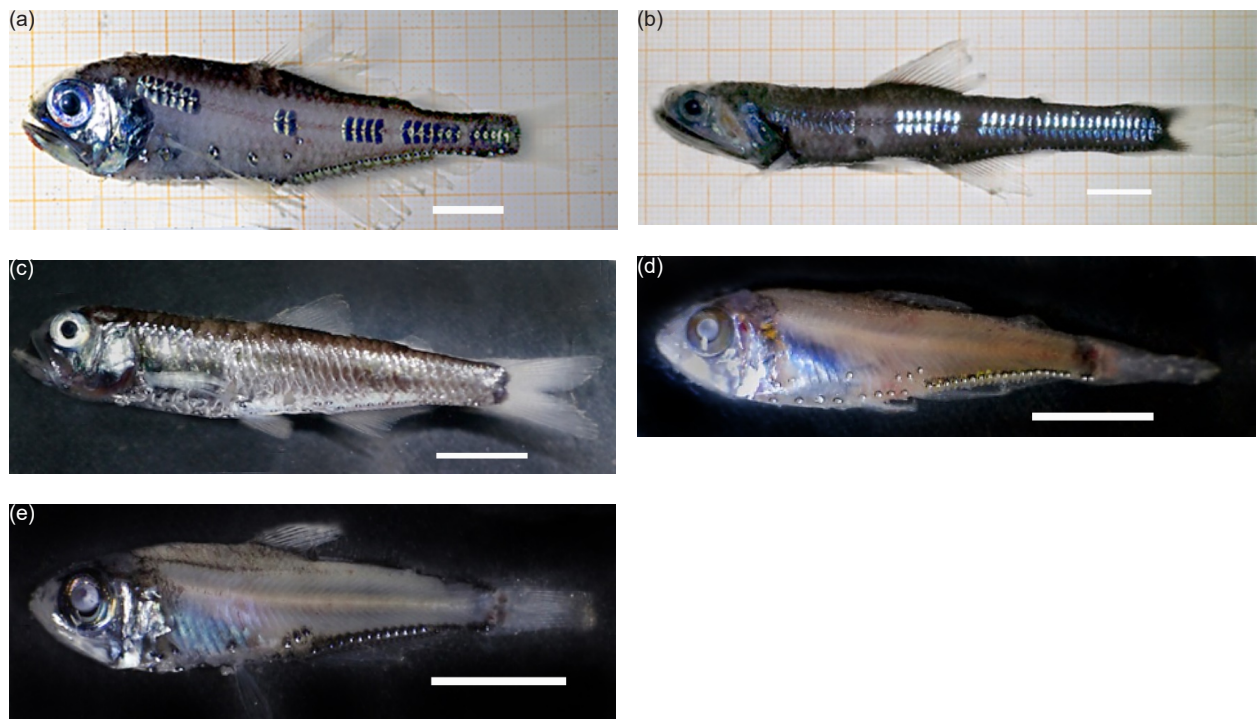


Figure 2: Lateral views of the studied Southern Ocean Myctophidae species: (a) *Electrona antarctica*, (b) *Gymnoscopelus braueri*, (c) *Krefftichthys anderssoni*, (d) *Protomyctophum tenisoni*, and (e) *Protomyctophum bolini*. Scale bar: 1 cm.

The two different stabilised storage forms of coelenterazine were also quantified in the samples. The first one, the enol-sulfate form was measured by taking 10 μl of the crushed sample, adding 100 μl of HCl 0.5 M and heating at 95°C for 1 min in order to hydrolyse the enol-sulfate group. The extract was then cooled on ice before adding NaHCO_3 and 90 μl of Tris-HCl buffer containing NaCl 0.15 M. Light emission was measured by adding a solution of 3 μl luciferase in 197 μl of Tris buffer 20 mM, NaCl 0.5 M. For the dehydrocoelenterazine form, 10 μl of the extract was mixed during 5 min with 0.5 mg of NaBH_4 in order to reduce the dehydrocoelenterazine to coelenterazine. Light emission was then measured by adding 3 μl of luciferase and 400 μl of Tris-HCl buffer 20 mM, NaCl 0.5 M. All the extractions were made using methanol saturated with argon and methanol alone (10 μl) was used as a negative control for each experimentation.

To measure luciferase activity, the sample was ground with 10 volumes of Tris-HCl buffer 10 mM, NaCl 0.15 M (pH 7.4), 20 and 40 μl of the extract were added to 180 and 160 μl of Tris-HCl buffer 10 mM, NaCl 0.5 M (pH 7.4) respectively. The luminescence reaction was initiated by the addition of 1 ml of Tris buffer containing 5 μl of a 1DO stock coelenterazine solution diluted at 1/200 (Prolume Ltd). Results reported are means of three measurements and are expressed in ng g^{-1} of fresh tissue for the coelenterazine content and in Megaquanta per second per gram of fresh tissue ($\text{Mq s}^{-1} \text{g}^{-1}$) for the luciferase activity. Light emissions were measured with a FB12 Berthold luminometer using fast kinetic software (FB12 Sirius Berthold detection system).

Results

In total, night trawls provided 14 different myctophid species with a total of 2 164 individuals. Only five species were present in large numbers (>100): *Electrona antarctica*, *Gymnoscopelus braueri*, *Krefftichthys anderssoni*, *Protomyctophum tenisoni* and *Protomyctophum bolini* (Figure 2). Specimens of these species were used to study bioluminescence.

Figure 3 illustrates *E. antarctica* with its ventral photophores under daylight (Figure 3a) and its corresponding bioluminescence (Figure 3b). Spectral analysis of bioluminescence (3C) revealed a peak value at $450 \pm 0.3 \text{ nm}$ that corresponds to

the known emission wavelength of natural coelenterazine (Shimomura, 1995). Coelenterazine and luciferase activity were detected in all the light organs of the five species of myctophids (Figures 4a to 4e). In photophores, the amount of coelenterazine varied widely from $14 \pm 13 \text{ ng g}^{-1}$ in *G. braueri* (Figure 4b) to $2\,975 \pm 575 \text{ ng g}^{-1}$ in *K. anderssoni* (Figure 4c), whereas luciferase activity displayed lesser variability with values ranging from $49 \pm 26 \text{ Mq s}^{-1} \text{g}^{-1}$ in *E. antarctica* (Figure 3a) to $493 \pm 370 \text{ Mq s}^{-1} \text{g}^{-1}$ in *P. tenisoni* (Figure 4e). Significant amounts of coelenterazine and luciferase activity were also found in all the tissues tested (skin, muscle, stomach, intestine, liver and gonads) of the five myctophids. Some species-specific variations were found, with important levels of both coelenterazine ($4\,177 \pm 2\,052 \text{ ng g}^{-1}$) and luciferase activity ($8\,054 \pm 6\,742 \text{ Mq s}^{-1} \text{g}^{-1}$) within the stomach tissue of *P. bolini* (Figure 4d), while *E. antarctica* showed very small amount of detected coelenterazine in the skin/muscle and gonadal tissues (Figure 4a). Although coelenterazine detection in the gonads varies widely, the compound was found in four of the five species. Very small amount of coelenterazine were detected in the skin/muscle tissue of all the studied species (Figures 4a to 4e). Finally, both the infra- and supra-caudal organs of *E. antarctica* and *K. anderssoni* showed significant quantities of coelenterazine as well as of luciferase activity (Table 1).

Coelenterazine storage forms were not systematically detected in all tissues of the five Myctophidae. Due to limited number of assays, only average values are provided; dehydrocoelenterazine was found in the five species but it was only weakly detected in muscle and skin tissues. Significant amounts were detected in photophores of *E. antarctica* and *P. tenisoni*, but the largest value (39 ng g^{-1}) was obtained from the liver of *G. braueri* (Table 2). The enol-sulfate derivative form was found in four of the five species: while *P. tenisoni* did not show its presence in any tissue, *P. bolini* displayed a huge amount of enol-sulfate derivative form in the liver ($2\,527 \text{ ng g}^{-1}$) (Table 3).

Taking into account the catch depths of the five studied myctophid species and their most common prey (data from Ivanov, 1970; Herring and Locket, 1978; Herring, 1988; Hulley, 1990; Haddock et al., 2010; Cherel et al., 2011; Saunders et al., 2015), it is clear that these fishes consume prey taxa known to use coelenterazine for their bioluminescence (Table 4).

Table 1: Mean values of coelenterazine concentration and luciferase activity measured in the supra- and infra-caudal organs of *E. antarctica* and *K. anderssoni*. N = number of replicates.

Species gland	Coelenterazine (in ng g ⁻¹)	N	Luciferase activity (in Mq s ⁻¹ g ⁻¹)
<i>E. antarctica</i> supra	207	2	240
<i>E. antarctica</i> infra	7	1	24
<i>K. anderssoni</i> supra	196	2	15
<i>K. anderssoni</i> infra	149	1	87

Table 2: Mean values of dehydrocoelenterazine stabilised storage form concentration found in several tissues for five myctophids; N = number of replicates.

Species	Tissue	N	Dehydrocoelenterazine (in ng g ⁻¹)
<i>E. antarctica</i>	Photophores	1	2.8
	Muscles and skin	1	0.06
<i>G. braueri</i>	Liver	1	39
	Muscles and skin	1	0.13
<i>K. anderssoni</i>	Muscles and skin	2	0.38
	Intestine	2	0.05
<i>P. bolini</i>	Muscles and skin	1	0.08
<i>P. tenisoni</i>	Photophores	3	2.3
	Intestine	3	2.3
	Muscles and skin	3	0.17

Table 3: Mean values of coelenterazine enol-sulfate stabilised storage form concentration found in several tissues for five myctophids; N = number of replicates.

Species	Tissue	N	Enol-sulfate form (ng g ⁻¹)
<i>E. antarctica</i>	Photophores	1	0.06
	Stomach	1	3.7
	Intestine	1	6.5
<i>G. braueri</i>	Photophores	1	3.3
	Intestine	1	23
	Skin	1	0.02
<i>K. anderssoni</i>	Liver	2	18
<i>P. bolini</i>	Liver	1	2 527
	Intestine	1	6.9
	Muscles and skin	1	0.01

Table 4: Catch depths of the five studied myctophid species and their most common prey; (*) indicates those prey taxa known to use coelenterazine for their bioluminescence (data compiled from Ivanov, 1970; Herring and Locket, 1978; Herring, 1988; Hulley, 1990; Haddock et al., 2010; Cherel et al., 2011; Saunders et al., 2015).

Species	Depth of capture (m)	Prey organisms
<i>Electrona antarctica</i>	55–150	Copepods*, amphipods, ostracods*, decapods*, euphausiids, polychaetes, fish juveniles
<i>Gymnoscopelus braueri</i>	55–150	Copepods*, amphipods, euphausiids
<i>Krefflichthys anderssoni</i>	580	Copepods*, euphausiids
<i>Protomyctophum bolini</i>	185–290	Copepods*, euphausiids
<i>Protomyctophum tenisoni</i>	270–520	Copepods*, ostracods*, euphausiids

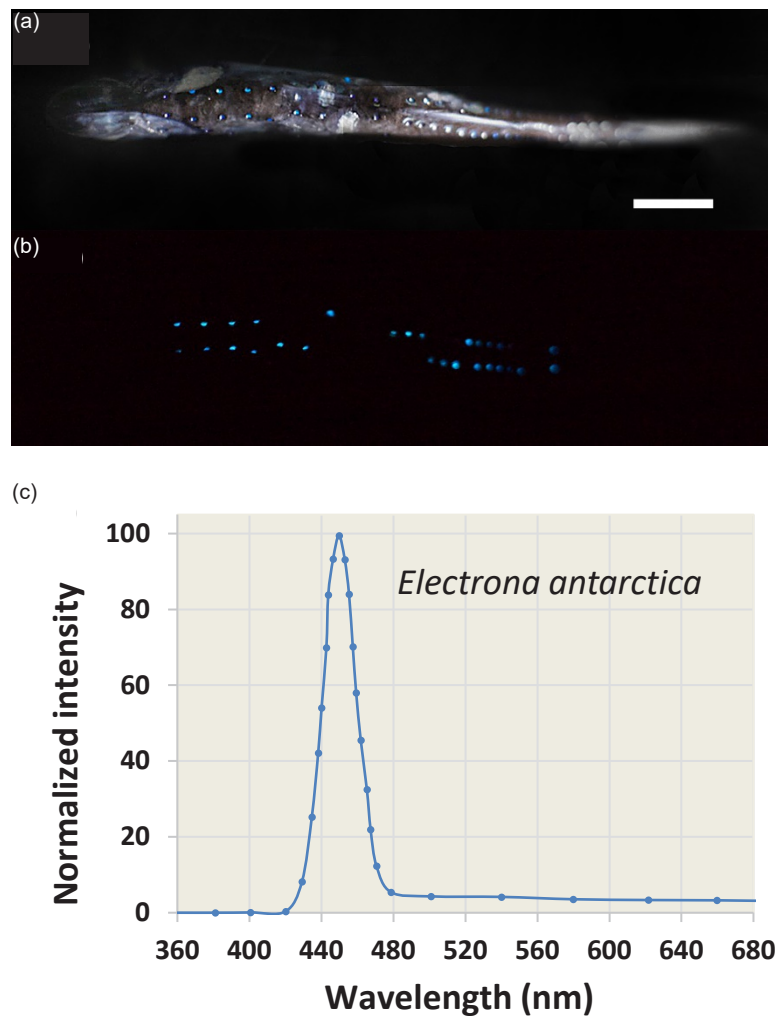


Figure 3: Ventral view of *Electrona antarctica* photophores in (a) daylight, (b) bioluminescence and (c) normalised spectrum of the light emission. Scale bar: 1 cm.

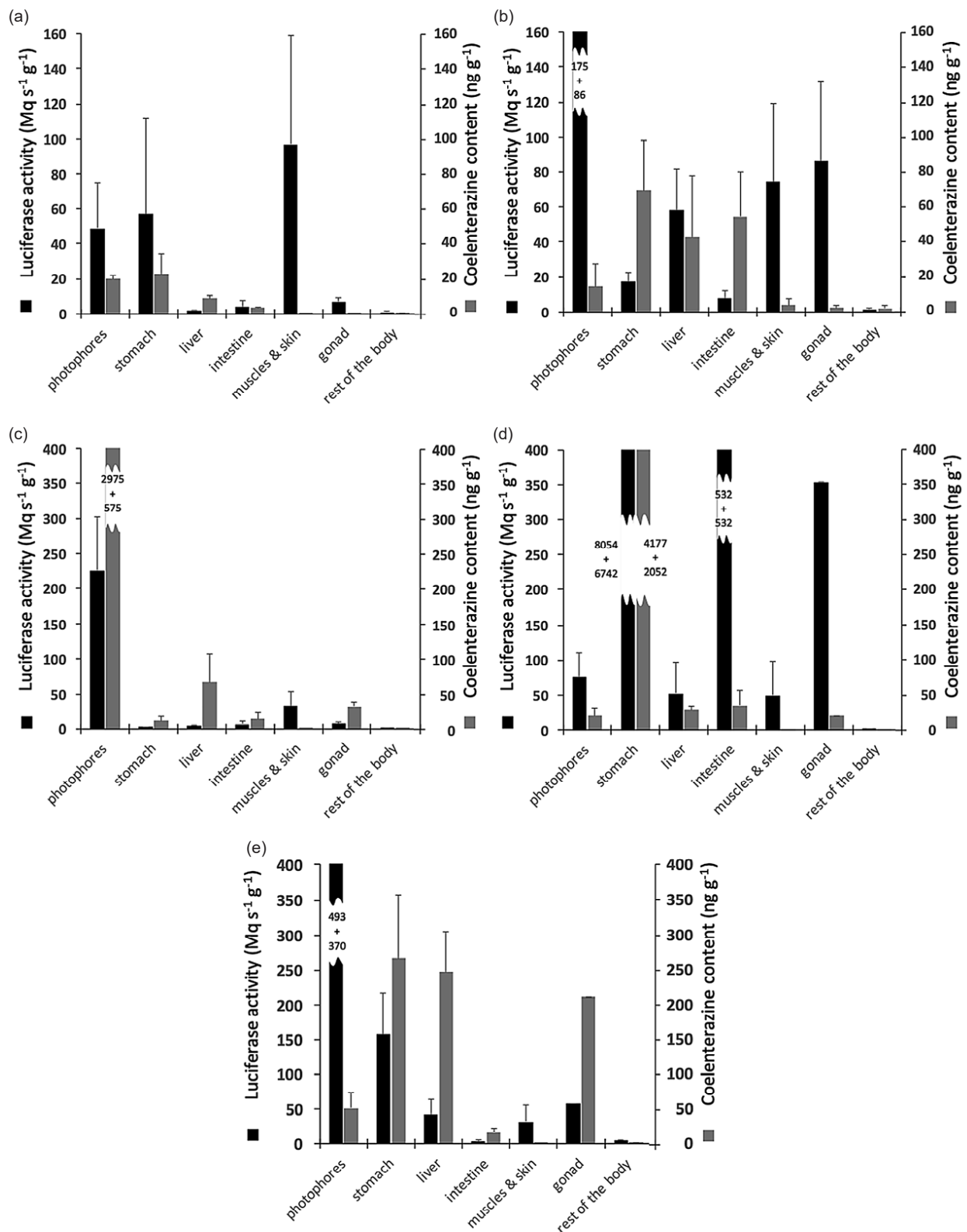


Figure 4: Mean values of luciferase activity (black bars, in $\text{Mq s}^{-1} \text{g}^{-1}$) and coelenterazine content (grey bars, in ng g^{-1}) of isolated tissues of five Southern Ocean myctophid species. (a) *E. antarctica*, (b) *G. braueri*, (c) *K. anderssoni*, (d) *P. bolini* and (e) *P. tenisoni*. $N = 3$ for each tissue. Values are means $\pm$ SE.

Discussion

Coelenterazine is a luminescent substrate found in nine different marine bioluminescent phyla (Cormier, 1978; Shimomura et al., 1980; Hastings and Morin, 1991; Haddock et al., 2010 for review). Our results add new support for the widespread occurrence of coelenterazine in mesopelagic fishes. The presence of derivative stabilised storage forms of coelenterazine in the liver, digestive tract, but also in other tissues of myctophids adds new clues to the hypothesis of the acquisition of the coelenterazine through the food web (Mallefet and Shimomura, 1995; Haddock et al., 2001). The list of prey consumed by the studied myctophids (Table 4), indicates the high number of organisms that are known to use coelenterazine to produce light. For example, Myctophidae are known to prey on luminous copepods (Takenaka et al., 2017). In a dietary study of Southern Ocean myctophids, the copepod *Metridia pacifica* was visually identified in the stomach content of the five species we studied (Saunders et al., 2015). Moreover, a recent metabarcoding dietary study using COI on myctophids from the Kerguelen Plateau revealed the presence of *M. pacifica* in stomachs of *G. braueri* (Clarke et al., 2018). *Metridia pacifica* can synthesise coelenterazine (Oba et al., 2009), thereby clearly supporting a dietary acquisition of coelenterazine by Myctophidae. This hypothesis assumes that coelenterazine is first ingested and absorbed, before being transferred either directly to photophores or stored in specific body compartments (e.g. liver) under its stabilised storage forms such as enol-sulfate and/or dehydrocoelenterazine (Cormier et al., 1970; Inoue et al., 1976; Takahashi and Isobe, 1994).

Detections of high level of coelenterazine concentration as well as of strong luciferase activity within the stomach and the digestive tract of some of the studied species, suggest that those specimens might have just fed on luminous organisms before capture. Variation of coelenterazine amounts within species and between species could therefore be explained by the quantity of recently ingested luminous prey (Young et al., 1979) and/or by the availability of storage forms within the tissue. In addition, this variation could also be due to differential physiological stress between specimens during trawling. All the results should be considered as minimum values since spontaneous decreases in coelenterazine levels and luciferase activities may occur during long-term storage, even in frozen conditions. Nevertheless, our results are in accordance

with previous studies supporting luciferin dietary acquisition in phylogenetically distant phyla (Tsuji et al., 1972; Frank et al., 1984; Harper and Case, 1999; Haddock et al., 2001).

In addition, our data confirmed those results obtained in previous studies where high amounts of coelenterazine were found within the different organs (liver, light organ) of Myctophidae. This phenomenon seems to characterise species from this family (Tsuji and Haneda, 1971; Inoue et al., 1977, 1979; Shimomura et al., 1980; Mallefet and Shimomura, 1995). Moreover, coelenterazine presence in the gonads of four of the five species tends to support the potential maternal transfer of the bioluminescence capability to the offspring. The detection of coelenterazine content and luciferase activity within the photophores and caudal organs clearly indicated that coelenterazine is the luminous substrate used in the light organs of Myctophidae.

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