Journal acronyn	n: TINV
Author(s):	Yudha Trinoegraha Adiputra, Muhammad Zairin jr., Muhammad Agus Suprayudi, Wasmen Manalu and Widanarni
Article title:	Identification of steroid hormones and fatty acids during gonadal maturation of spiny lobster <i>Panulirus homarus</i>
Article no:	1549114
Enclosures:	1) Query sheet 2) Article proofs

PROOF COVER SHEET

Dear Author,

1. Please check these proofs carefully. It is the responsibility of the corresponding author to check these and approve or amend them. A second proof is not normally provided. Taylor & Francis cannot be held responsible for uncorrected errors, even if introduced during the production process. Once your corrections have been added to the article, it will be considered ready for publication.

Please limit changes at this stage to the correction of errors. You should not make trivial changes, improve prose style, add new material, or delete existing material at this stage. You may be charged if your corrections are excessive (we would not expect corrections to exceed 30 changes).

For detailed guidance on how to check your proofs, please paste this address into a new browser window: http://journalauthors.tandf.co.uk/production/checkingproofs.asp

Your PDF proof file has been enabled so that you can comment on the proof directly using Adobe Acrobat. If you wish to do this, please save the file to your hard disk first. For further information on marking corrections using Acrobat, please paste this address into a new browser window: http://journalauthors.tandf.co.uk/production/acrobat.asp

2. Please review the table of contributors below and confirm that the first and last names are structured correctly and that the authors are listed in the correct order of contribution. This check is to ensure that your name will appear correctly online and when the article is indexed.

Sequence	Prefix	Given name(s)	Surname	Suffix
1.		Yudha Trinoegraha	Adiputra	
2.		Muhammad	Zairin jr.	
3.		Muhammad Agus	Suprayudi	
4.		Wasmen	Manalu	
5.				

Queries are marked in the margins of the proofs, and you can also click the hyperlinks below.

Content changes made during copy-editing are shown as tracked changes. Inserted text is in red font and revisions have a red indicator \checkmark . Changes can also be viewed using the list comments function. To correct the proofs, you should insert or delete text following the instructions below, but **do not add comments to the existing tracked changes.**

AUTHOR QUERIES

General points:

- 1. **Permissions:** You have warranted that you have secured the necessary written permission from the appropriate copyright owner for the reproduction of any text, illustration, or other material in your article. Please see http://journalauthors.tandf.co.uk/permissions/usingThirdPartyMaterial.asp.
- 2. **Third-party content:** If there is third-party content in your article, please check that the rightsholder details for re-use are shown correctly.
- 3. **Affiliation:** The corresponding author is responsible for ensuring that address and email details are correct for all the co-authors. Affiliations given in the article should be the affiliation at the time the research was conducted. Please see http://journalauthors.tandf.co.uk/preparation/writing.asp.
- 4. **Funding:** Was your research for this article funded by a funding agency? If so, please insert 'This work was supported by <insert the name of the funding agency in full>', followed by the grant number in square brackets '[grant number xxxx]'.
- 5. Supplemental data and underlying research materials: Do you wish to include the location of the underlying research materials (e.g. data, samples or models) for your article? If so, please insert this sentence before the reference section: 'The underlying research materials for this article can be accessed at <full link> / description of location [author to complete]'. If your article includes supplemental data, the link will also be provided in this paragraph. See

<http://journalauthors.tandf.co.uk/preparation/multimedia.asp> for further explanation of supplemental data and underlying research materials.

- 6. The **CrossRef database** (www.**crossref**.org/) has been used to validate the references. Changes resulting from mismatches are tracked in red font.
- AQ1 Please check whether check Author Widanarni name set correctly.
- AQ2 Abstract must be maximum of 200 words long. Please check.
- **AQ3** Abbreviations are allowed as per journal style. Please check and provide if any.
- AQ4 The funding information "Doctoral Dissertation Research Grant from Ministry of Research, Technology and Higher Education of The Republic of Indonesia" provided has been checked against the Open Funder Registry and we failed to find a match. Please confirm if the Funding section is accurate and also confirm the funder names "Doctoral Dissertation Research Grant from Ministry of Research, Technology and Higher Education of The Republic of Indonesia".
- AQ5 Please check Acknowledgments can be changed into funding.
- AQ6 Please check the spelling of the word "cyclopropentanoic acid". Please provide correct spelling if necessary in all occurrences..
- AQ7 Please check the spelling of the word "prepenoic acid". Can this be changed to "propenoic acid?". Please provide correct spelling if necessary in all occurrences..
- AQ8 Please check the spelling of the word "nonadecanoid acid". Can this be changed to "nonadecanoic acid?". Please provide correct spelling if necessary in all occurrences..
- AQ9 Please check the spelling of the word "cyclopentaneundecanoid acid". Can this be changed to "cyclopentaneundecanoic acid?".Please provide correct spelling if necessary in all occurrences in all occurrences..

- AQ10 Please check the spelling of the word "pentadecanoid acid". Can this be changed to "pentadecanoic acid?". Please provide correct spelling if necessary in all occurrences..
- AQ11 Please check the spelling of the word "octadecanoid acid". Can this be changed to "octadecanoic acid?". Please provide correct spelling if necessary in all occurrences..
- AQ12 The disclosure statement has been inserted. Please correct if this is inaccurate.
- AQ13 The CrossRef database (www.crossref.org/) has been used to validate the references. Mismatches between the original manuscript and CrossRef are tracked in red font. Please provide a revision if the change is incorrect. Do not comment on correct changes.

How to make corrections to your proofs using Adobe Acrobat/Reader

Taylor & Francis offers you a choice of options to help you make corrections to your proofs. Your PDF proof file has been enabled so that you can mark up the proof directly using Adobe Acrobat/Reader. This is the simplest and best way for you to ensure that your corrections will be incorporated. If you wish to do this, please follow these instructions:

1. Save the file to your hard disk.

2. Check which version of Adobe Acrobat/Reader you have on your computer. You can do this by clicking on the "Help" tab, and then "About".

If Adobe Reader is not installed, you can get the latest version free from http://get.adobe.com/reader/.

3. If you have Adobe Acrobat/Reader 10 or a later version, click on the "Comment" link at the right-hand side to view the Comments pane.

4. You can then select any text and mark it up for deletion or replacement, or insert new text as needed. Please note that these will clearly be displayed in the Comments pane and secondary annotation is not needed to draw attention to your corrections. If you need to include new sections of text, it is also possible to add a comment to the proofs. To do this, use the Sticky Note tool in the task bar. Please also see our FAQs here: http://journalauthors.tandf.co.uk/production/index.asp.

5. Make sure that you save the file when you close the document before uploading it to CATS using the "Upload File" button on the online correction form. If you have more than one file, please zip them together and then upload the zip file.

If you prefer, you can make your corrections using the CATS online correction form.

Troubleshooting

Acrobat help: http://helpx.adobe.com/acrobat.html Reader help: http://helpx.adobe.com/reader.html

Please note that full user guides for earlier versions of these programs are available from the Adobe Help pages by clicking on the link "Previous versions" under the "Help and tutorials" heading from the relevant link above. Commenting functionality is available from Adobe Reader 8.0 onwards and from Adobe Acrobat 7.0 onwards.

Firefox users: Firefox's inbuilt PDF Viewer is set to the default; please see the following for instructions on how to use this and download the PDF to your hard drive:

 $http://support.mozilla.org/en-US/kb/view-pdf-files-firefox-without-downloading-them \#w_using-a-pdf-reader-pluging-them \#w_using-a-pdf-reader-pluging-a-pdf$



Identification of steroid hormones and fatty acids during gonadal maturation of spiny lobster *Panulirus homarus*

Yudha Trinoegraha Adiputra^a, Muhammad Zairin jr.^a, Muhammad Agus Suprayudi^a, Wasmen Manalu^b and Widanarni^a

AQ1

^aDepartment of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia; ^bDepartment of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, Indonesia

ABSTRACT

Information on steroid hormones and fatty acids that play roles in lobster reproduction is still very limited although the data are indispensable to seed production in hatchery. The study was 10 designed to identify steroid hormones and fatty acids during gonadal maturation of spiny lobster (Panulirus homarus). Pyrolysis GCMS was used to identify steroid hormones and fatty acids and compared their concentrations between sexes and treatments. Samples from 6 male and 18 female spiny lobsters were used with different treatments. Male spiny lobsters were treated with and without thyroxine injection. Female spiny lobsters were treated with and without eyestalk 15 ablations during mature and immature gonad developments. Androst-5-en-17-one,3ß (androst) and estran-3-one, 17β (estran), two steroid hormones were identified at different levels of gonadal maturity of spiny lobsters. High concentrations of androst and estran were detected in the male spiny lobsters treated with thyroxine injections. Estran has limited roles in stimulating gonadal development in male spiny lobsters. Androst was also found in the gonad of mature female spiny 20 lobsters, but with low concentrations. Estran showed high concentrations in female brood stock of spiny lobsters during oogenesis stages both without eyestalk ablation and with ablation of one or two eyestalks, except in the immature female gonads. It was found that stearic acid was the highest and dominant fatty acid in mature male spiny lobster. Stearic acid, oleic acid, palmitic acid and caprylic acid were fatty acids with high concentrations in immature and mature female spiny lobsters. After 30 days in captivity, only stearic acid and oleic acid were found dominantly in eyestalk ablated mature female spiny lobsters.

25 AQ2

Introduction

Reproduction in crustaceans is controlled by endocrine factors and nutritional conditions (Subramoniam 2011).

- 30 The roles of hormones and fatty acids in reproduction are reflected in gonadal maturation technology that has been applied practically in hatcheries to produce post larvae (Wilder et al. 2010). Eyestalk ablation is a conventional technique applied to male and female spiny lobsters
- 35 (*Panulirus homarus*) to accelerate gonad maturity, increase feed intake, and its conversion related to reproduction, and more specifically to shorten the duration from intermoult to premoult stages (Radhakrishnan and Vijayakumaran 1984; Vijayakumaran and Radhakrishnan
- 40 1984; Fernandez and Radhakhrisnan 2016).

Another gonadal maturation technology is the injection of sex steroids hormones of vertebrates. Kirubagaran et al. (2005) reported two steroid hormones namely estradiol and progesterone during ovarian maturation in spiny

45 lobster. The steroid hormones play roles in gonadal

maturation, shorten maturation period, increase matured female, and extend the periods of fertilisation in spiny lobsters (*P. interruptus*) (Nan et al. 2015).

Nandi (1967) explained that the quantitative and qualitative analysis of hormones and the release of 50 hormones from the gland were the activities that can link the important functions of steroid hormone and nutrition in reproduction. Nutrients such as lipid, protein, carbohydrate, vitamin and minerals are important factors for maturation process of crustacean (Harrison 55 1990). Lipid is a major energy source in marine invertebrate, involved in many important processes including growth, moult and reproduction (Yan et al. 2017). Nutrients requirements of brood stock can be traced on the basis of changes in the composition of the 60 corresponding materials when the maturation process occurs in the crustacean gonads (Harrison 1990). Lack of information on steroid hormone and fatty acids in spiny lobster gonadal maturation is due to the limited utilisation of this species in commercial mariculture, 65

CONTACT Muhammad Zairin jr. Szirinmz@live.com Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University Agatis Street, Dramaga Campus, Bogor, West Java 1680, Indonesia

ARTICLE HISTORY

Received 9 August 2018 Accepted 12 November 2018

KEYWORDS

Eyestalk ablation; fatty acid; spiny lobster; steroid hormone; thyroxine injection

AQ3

especially in hatchery. This study was designed to identify the types and levels of steroid hormones and fatty acids during gonadal maturation in spiny lobster.

Materials and methods

70 Spiny lobster origin and rearing

Immature and mature male and female spiny lobsters used in the experiment were obtained from Krui, West Coast Residence, Lampung Province, Indonesia. Mature male spiny lobsters with body weights range of 145–

- 75 152 g, immature female spiny lobsters with body weights range of 126–134 g, and mature female spiny lobsters with body weights range of 148–174 g were handled with sea sand, ice, and packed in paper box, and then transported by car about six hours to Main
- 80 Center for Marine Aquaculture or MCMA (Balai Besar Perikanan Budidaya Laut) in Pesawaran Residence, Lampung Province, Indonesia. Permit clearance was obtained from Fish Quarantine and Fish Quality Products Inspection Office of Lampung Province to
- 85 use spiny lobsters for research purposes. Four fibres illuminated plastic tanks each with the size of 200 × 100×50 cm were used for rearing of spiny lobsters. The tanks were filled with sea water with 40 cm height, continuously changed, and aerated. Fresh squid or fish
- 90 meats were used as feeds given twice a day at 08 am and 05 pm with the level of 3–5% of body weight. The experimental tanks were siphoned and cleaned two times daily at 07 am and 04 pm. For a shelter of spiny lobster in captivity, each tank was provided with 10 PVC
- 95 pipes each with 6-inch diameter and 30 cm length.

Spiny lobster treatments

Spiny lobster of 24 individuals consisting of 6 males and 18 females were used in the experiment. Mature male spiny lobsters, each with body weight of 145–152 g, 100 were identified or marked individually by the number written on the paper covered with transparent waterproof plastic and tighten into the tail by rubber band. All experimental male spiny lobsters were reared in one tank. The observations for male spiny lobsters were

105 divided into two parts.

110

Part I. The Effect of Thyroxine Injection on Gonadal Maturation of Male Spiny Lobsters

Two mature male spiny lobsters were used to study the effect of thyroxine hormone injection on gonadal maturation. The dosages of thyroxine hormone used were 0 and 0.1 µg/g body weight. Two experimental male spiny lobsters were used in this test (each dose of

thyroxine injection only used one male spiny lobster). Levothyroxine sodium tablets (Thyrax N.V. Organon, Oss. The Netherlands) with thyroxine concentration of 115 100 µg in one tablet was used as a source of thyroxine hormone in this study. Stock solution for thyroxine hormone injection was made by crushing one tablet of Thyrax into powder form and dissolved in 100 µL of physiological NaCl (0.9 g NaCl/100 µL sterile ddH₂O) as 120 a stock solution and then a serial dilution was made to obtain the required concentrations. Thyroxine hormone at a dose of 0 µg/g was used 1 mL of physiological NaCl for injection without thyroxine. Gonads from two male spiny lobsters injected with thyroxine at doses of 0 and 125 0.1 μ g/g body weights, respectively, were collected β days after thyroxine injections.

Part II. The Effect of Thyroxine Injection on the Growth of Male Spiny Lobster

Four gonad mature male spiny lobsters were used to 130 study the effect of thyroxine hormone injection on the growth of male spiny lobster. The doses of thyroxine injection used were 0; 0.1; 0.2; and 0.5 μ g/g body weight. Thyroxine hormone stock solution and injection doses were similar with those used in Part I above. Gonads 135 from four male spiny lobsters injected with thyroxine at doses of 0; 0.1; 0.2; and 0.5 μ g/g body weights were isolated eight days after thyroxine injection.

A total of 18 female spiny lobsters were used and the treatments were divided into three parts.

Part I. Effect of one eyestalk ablation on immature female spiny lobster

The first part consisted of six immature female spiny lobsters with body weight range of 122–134 g. The experimental female spiny lobsters were divided into two treatments i.e., without eyestalk ablation and with one eyestalk ablation. Each treatment consisted of three immature female spiny lobsters.

Part II. Effect of one eyestalk ablation on mature female spiny lobster

The second part consisted of six mature spiny lobsters with body weight range of 148–152 g. The mature female spiny lobsters were divided into two treatments i.e., without eyestalk ablation and with one eyestalk ablation. Each treatment consisted of three mature female spiny lobsters.

Part III. Effect of one and two eyestalk ablation on mature female spiny lobster

The mature spiny lobsters were treated with two stages of treatment of eyestalk ablation. This part of experiment used six matured female spiny lobsters with body

150

155

140

weight range of 163-174 g. In the beginning of the experiment, or on day one of treatment, all of six experimental matured female spiny lobsters were eye-

- stalk ablated. After 30 days of one eyestalk ablation 165 (on day 31), three of experimental mature female spiny lobsters previously ablated with one eyestalk were continued with the ablation of the other eyestalk to obtain a two eyestalk ablation.
- 170 All matured female groups of experimental spiny lobsters were reared in three different tanks and marking methods were used similar to those used in male spiny lobster. From the first day of rearing or maintenance, the gonad was taken by following the changes
- in the three stages of oogenesis. Practically, the condi-175 tion and stage of oogenesis phases was confirmed by bending the cephalothorax of female spiny lobster and highlighting it with a flask light. The changes in the colour of the gonads were main indicators used to
- determine the phases of oogenesis. After the gonad 180 reached the oogenesis, the spiny lobster was dissected without considering the duration of maintenance to reach gonad maturity. In female spiny lobsters without eyestalk ablation that were difficult to reach peak of oogenesis phases, feeding level was increased to 8% of 185
- body weight/day and duration of rearing was extended to 14 days.

Ovaries were taken in each phase of three phases of oogenesis in three groups of experimental female spiny

190 lobsters. Three phases of oogenesis were primary vitellogenesis, secondary vitellogenesis and maturation that were confirmed by the histology of ovaries. Determination of three phases of oogenesis and histological analysis were conducted by following the methods of Subramoniam (2017a) and Shields and 195 Boyd (2014) (Figure 1). Clove oil at a dose of 10 ml/L of sea water was used as an anaesthetic agent. Eyestalk ablation was conducted by cutting the eyestalk by using a sterile scalpel and sanitised the cut eyestalk with iodine to protect from pathogens infection. Gonad 200 samples from male and female groups of experiments were kept in -80°C until analysis with a pyrolysis GCMS.

Pyrolysis gas chromatography mass spectrometry analysis

Approximately 1 µg of gonad was weighed with micro-205 balance and pyrolysed at 400°C. The products of pyrolysis were analysed by GCMS. Gas Chromatography separations were carried out with a GCMS-QP2010 (Shimadzu, Tokyo) plus instrument equipped with an rt × 5 ms capillary column (length of 60 cm, diameter of 210 0.25 mm, and thickness of 0.25µm). Chromatographic separation was achieved by the following temperature program: 50°C for 5 min, then it was raised to 280°C for 50 min with pressure of 101 kPa. Helium was the carrier gas at 0.85 mL/min with total flow of 46.5 mL/min and 215 flow program mode at a linear velocity of 23.7 cm/sec, and purge flow of 3.0 mL/min. Split injection mode used was the ratio of 1:50. Mass spectra was set with ion source at 200°C and interface at 280°C with solvent cut time of 1.5 min. Detector temperature used was 220 280°C and compound identification was based on comparison of mass spectra with WILEY7 library database. Compounds with the highest similarity (>90%) were

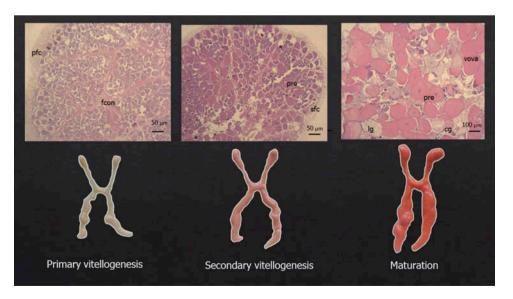


Figure 1. Oogenesis stages of spiny lobster (Panulirus homarus) ovaries used in this study. pfc: primary follicular cell; fcon: fibrous connective tissue; sfc: secondary follicular cell; pre: previtellogenic oocyte; lg: lipid globule; vova: vitellogenic oocyte; cg: cortical granule.

225

identified as steroid hormones and fatty acids data were selected from five ranks of compounds similarity available from library database.

Data analyses

WILEY7 library database was able to identify structural and molecular weights of steroid hormones and fatty acids. Due to the focus on steroid hormones and fatty 230 acids identification, two treatments on male spiny lobster used were with and without thyroxine injection. It means the effects of thyroxine injection on growth and gonadal maturation and their doses of injection were ignored. 235 Identified steroid hormones that appeared more than

one concentration were presented as mean \pm SD.

Fatty acids identifications were conducted, due to their roles as main nutrients involved in gonadal maturation. Further, comparisons of concentrations of those

240 above fatty acids were conducted between male and female gonads and between treatments. Fatty acids that appeared more than one concentration were presented as mean \pm SD. In order to compare similar fatty acids among treatments with exception to steroid hor-

245 mones, concentration was grouped into three categories i.e., high (>10%), moderate (5–10%), and low (<5%).

Results

2.50

Pyrolysis GCMS showed the ability to identify steroid hormones and fatty acids during gonadal maturation of spiny lobsters. Two steroid hormones found in this study were androst-5-en-17-one,3β (androst) and estran-3-one,17β (estran). Pyrolysis GCMS also provided structures and molecular weights of these two steroids hormones (Figure 2).

During gonadal maturation of male spiny lobsters, 255 androst showed a high concentration in control spiny lobsters without thyroxine injection (2.00 \pm 0.09%) compared to those injected with thyroxine (0.31 \pm 0.00%). Estran was also found in gonadal mature male spiny lobsters without thyroxine injection with concentrations 260 of 0.74 \pm 0.00% and 1.00 \pm 0.07% in mature male spiny lobsters with thyroxine injection (Table 1).

Concentrations of estran decreased in immature female spiny lobsters with one eyestalk ablation and without eyestalk ablation in different stages of oogen-265 esis. Without eyestalk ablation, the immature female spiny lobsters showed an inconsistent decrease in estran concentrations within different stages of oogenesis i.e., primary vitellogenesis (8.14 \pm 0.50%), secondary vitellogenesis (3.74 \pm 0.69%), and maturation (4.88 \pm 0.57%). 270 However, immature female spiny lobsters with one evestalk ablation also showed a consistent decrease in the concentrations of estran in different stages of oogenesis i.e., primary vitellogenesis (13.79 ± 2.59%), secondary vitellogenesis (4.61 ± 1.49%), and maturation stage 275 $(3.26 \pm 0.66\%)$ (Table 1). Androst was not detected in immature female spiny lobster.

Different from the immature female spiny lobster, high concentrations of estran and androst were detected in mature female spiny lobsters. Mature female spiny 280 lobsters without eyestalk ablation showed a pattern of increase in estran concentrations with the stage of oogenesis i.e., primary vitellogenesis (0.13 \pm 0.00%), secondary vitellogenesis (5.30 ± 0.00%), and maturation stage (5.56 \pm 0.81%). Mature female spiny lobster with 285 one eyestalk ablation also showed an increase in estran from 2.35 ± 0.66% during primary vitellogenesis to $10.86 \pm 3.10\%$ during maturation stage. In this group of mature female spiny lobster with one eyestalk ablation, during secondary vitellogenesis, estran and androst 290

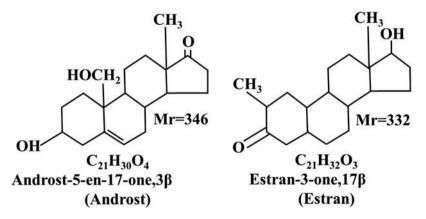


Figure 2. Structures and molecular weights of androst-5-en-17-one,3ß (androst) and estran-3- one,17ß (estran). Two steroid hormones in immature and mature gonads of male.

diffe	different treatments.	ments.						
No	Sexes	Treatments	Conc	Concentration of androst (%)		Conc	Concentration of estran (%)	
- 4	Male Male	Without thyroxine injection Thyroxine injection		2.00 ± 0.09 0.31 ± 0.00			0.74 ± 0.00 1.00 ± 0.07	
			Concentration of	Concentration of androst (%) during oogenesis phases	is phases	Concentration of	Concentration of estran (%) during oogenesis phases	phases
			Primary vitellogenesis	Primary vitellogenesis Secondary vitellogenesis	Maturation	Primary vitellogenesis	Primary vitellogenesis Secondary vitellogenesis	Maturation
m	Female	Female Immature and without eyestalk ablation	Not detected	Not detected	Not detected	8.14 ± 1.50	3.74 ± 0.69	4.88 ± 0.57
4	Female	Immature and one eyestalk ablation	Not detected	Not detected	Not detected	13.79 ± 2.59	4.61 ± 1.49	3.26 ± 0.66
S	Female	Mature and without eyestalk ablation	Not detected	Not detected	Not detected	0.13 ± 0.00	5.30 ± 0.00	5.56 ± 0.81
9	Female	Mature and one eyestalk ablation	Not detected	0.37 ± 0.00	Not detected	2.35 ± 0.66	1.37 ± 0.00	10.86 ± 3.10
7	Female	Mature and one eyestalk ablation after 30 days in captivity	0.84 ± 0.00	Not detected	1.11 ± 0.00	2.59 ± 0.68	3.93 ± 0.94	5.17 ± 1.47
8	Female	Mature and two eyestalk ablation after 30 days in captivity	Not detected	Not detected	Not detected	2.52 ± 0.00	7.07 ± 1.63	7.85 ± 1.73

.⊆

Table 1. Two steroid hormones androst-5-en-17-one,38 (androst) and estran-3-one,178 (estran) concentration (%) during gonadal maturation of spiny lobster (Panulirus homarus)

showed low concentrations i.e., 1.37 \pm 0.00% and $0.37 \pm 0.00\%$, respectively (Table 1).

Matured female spiny lobsters with one and two eyestalk ablation after 1 month in captivity and three stages of oogenesis showed variations of estran and 295 androst concentrations. Mature female spiny lobsters with one eyestalk ablation showed increases in the concentrations of estran with the stages of oogenesis i.e., primary vitellogenesis (2.59 \pm 0.00%), secondary vitellogenesis $(3.93 \pm 0.94\%)$, and maturation stage 300 (5.17 \pm 1.47%). Concentrations of androst were also detected to increase from primary vitellogenesis $(0.84 \pm 0.00\%)$ to maturation stage $(1.11 \pm 0.00\%)$. Twoeyestalk ablated mature female spiny lobsters showed the increase in estran concentrations during oogenesis 305 stages i.e., primary vitellogenesis (2.52 ± 0.00%), secondary vitellogenesis (7.07 \pm 1.63%), and maturation stage (7.85 ± 1.73%) (Table 1).

Variations of fatty acids concentrations were detected during gonadal maturation of spiny lobster treated without 310 thyroxine injection and with thyroxine injection. Results of experiment showed that deposition of fatty acids in the gonads of mature male spiny lobsters was dominated by stearic acid with a high concentration (13.05 \pm 2.37%; 10.84 \pm 1.13%). Fatty acids with low concentrations in this 315 treatment were oleic acid (2.83 \pm 0.55%), pentadecanoic acid (1.80 ± 0.00%), cyclopropane pentanoic acid $(1.49 \pm 0.00\%)$, hexyl oleate $(1.35 \pm 0.00\%)$, and docosaenoic acid (1.83 ± 0.00%) (Table 2).

In immature female spiny lobsters without eyestalk 320 ablation, fatty acids found in high concentrations were stearic acid (16.47 ± 0.00; 22.32 ± 0.00%), oleic acid $(25.96 \pm 4.31\%)$, and palmitic acid $(11.33 \pm 0.00\%)$. Octadecatrienoic acid (2.60 \pm 0.00%), cyclopropentanoic acid (2.59 \pm 0.00%), prepenoic acid (1.15 \pm 0.00%), pen-325 tadecanoic acid (0.80 ± 0.00%), nonadecanoid acid (1.15 \pm 0.00%), and cyclopentaneundecanoid acid $(0.59 \pm 0.00\%)$ were identified with low concentrations. Fatty acids with high concentrations in immature female spiny lobster with one eyestalk ablation were caprylic 330 acid (24.08 \pm 0.00%) and oleic acid (10.90 \pm 0.85; $27.08 \pm 8.74\%$). Low concentration in immature female spiny lobster with one eyestalk ablation were docosenoic acid (4.89 \pm 0.00%), hexanoic acid (1.34 \pm 0.00%), and undecenoic acid $(1.11 \pm 0.00\%)$ (Table 3). 335

In mature female spiny lobsters without eyestalk ablation, fatty acids deposition showed a great variation. Fatty acids that were found with high and moderate concentrations during secondary vitellogenesis and maturation stages were cyclopentaneundecanoid acid 340 $(15.62 \pm 0.00\%)$, stearic acid $(9.63 \pm 0.00; 16.82 \pm 0.00\%)$, and oleic acid (12.35 ± 0.00%). Fatty acids with low concentrations were octadecanoic acid (4.50 \pm 0.53%),

AQ9

AQ6

AQ7

AQ8

Table 2. Identified fatty acids concentration (%) during gonadal maturation of male spiny lobster (Panulirus homarus).

No	Treatments (number of sample)	Body weight (g)	Maturity	Identified fatty acids	Concentration (%)
1	Without thyroxine injection (2)	145;150	Mature	Octadecanoid acid/Stearic acid	13.05 ± 2.37
				Pentadecanoid acid,14-methyl-,methyl ester	1.80 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	1.49 ± 0.00
				9-Octadecenoid acid, hexyl ester/Hexyl oleate	1.35 ± 0.00
				13-Docosenoic acid, methyl ester	1.03 ± 0.00
2	Thyroxine injection (4)	148–152	Mature	Octadecanoid acid/Stearic acid	10.84 ± 1.13
				9-Octadecenoid acid/Oleic acid	2.83 ± 0.55

- prepenoic acid (1.32 ± 0.00%), docosenoic acid 345 $(0.79 \pm 0.00\%)$, and butanoic acid $(1.53 \pm 0.00\%)$. Fatty acids with moderate and low concentrations during primary vitellogenesis were palmitic acid $(5.78 \pm 0.00\%)$, oleic acid $(4.98 \pm 0.00\%)$, ethyl palmitate $(3.32 \pm 0.00\%)$, and prepenoic acid $(0.07 \pm 0.00\%)$ (Table 3). Mature female spiny lobsters with one eyestalk abla-350
- tion showed a variation. In this group of mature female spiny lobsters with one eyestalk ablation, concentrations of palmitic acid (15.55 \pm 0.00; 10.12 \pm 0.00; 17.24 ± 0.00%), cyclopentaneundecanoid acid
- 355 $(13.72 \pm 0.00\%)$, and oleic acid $(11.39 \pm 0.00\%)$ were dominantly high. In this group of mature female spiny lobsters with one eyestalk ablation, fatty acids showing moderate concentrations were cyclopropane pentanoic acid (5.40 \pm 0.61%), eicosatrienoic acid (4.20 \pm 0.00%),
- and decanoic acid (5.33 \pm 0.00%). In this group of 360 mature female spiny lobsters with one eyestalk ablation, fatty acids with low concentrations during secondary vitellogenesis and maturation stage were AQ10 pentadecanoid acid (2.44 ± 0.83%), octadecanoid acid (1.62 \pm 0.00%), and prepenoic acid (0.38 \pm 0.00; 365

0.44 ± 0.00%) (Table 3).

- AQ11
 - After one month in captivity, mature female spiny lobsters with one and two eyestalk ablations showed distinctly different fatty acids concentrations. In mature
 - 370 female spiny lobsters with one and two eyestalk ablations, fatty acids showing high concentrations were stearic acid (17.78 ± 0.00; 15.81 ± 0.00; 11.04 ± 0.00; 14.39 \pm 0.00%) and oleic acid (16.74 \pm 0.00; 18.80 ± 0.00 ; $13.01 \pm 0.00\%$) followed by a variety of
 - 375 moderate and low concentrations of fatty acids. In contrast, fatty acids during maturation stage were dominated only by stearic acid 16.45 ± 0.00 ; $11.95 \pm 0.00\%$) and oleic acid (9.77 \pm 0.00; 11.33 \pm 0.00%) and no other fatty acid was detected during maturation stage. In
 - 380 mature female spiny lobsters with one and two eyestalk ablations, fatty acids showing moderate and low concyclopropanepentanoic centrations were acid $(8.74 \pm 0.00; 4.98 \pm 1.23; 4.53 \pm 0.75)$, octadecatrienoic acid (1.33 \pm 0.00; 2.33 \pm 0.00), pentadecanoic acid $(1.32 \pm 0.00; 2.86 \pm 0.00)$, and prepenoic acid 385
 - (1.45 ± 0.00) (Table 3).

Discussion

Analytical platform of GCMS has advantages and disadvantages in determining metabolites samples (Young and Alfaro 2018). The combination between 390 platforms such as pyrolysis GCMS may support more advantages that can be used to obtain broad analysis coverage including hormones and fatty acids identifications with very limited sample numbers. The use of pyrolysis GCMS is robust and convenient to produce 395 the results rapidly. Pyrolysis GCMS has been applied for exploring the natural active compounds from biological materials (Martinez-Balmori et al. 2013). The use of pyrolysis GCMS is able to find new products (Ghalibaf et al. 2017). This study proved the benefits 400 of pyrolysis GCMS in identification of steroid hormones and fatty acids in different stages of gonad maturation and treatments.

Hormonal regulation of reproduction in spiny lobster is debatable due to the limited studies previously con-405 ducted to improve spiny lobster hatchery production (Subramoniam 2017b). Progesterone and 17β-estradiol are hormones recorded to affect vitellogenesis (Subramoniam and Kirubagaran 2010). This present study found two new steroid hormones that had roles 410 in gonadal maturation and vitellogenesis of male and female spiny lobsters that were different from vertebrate-type sex steroid hormones published so far. In general, steroids are available in two main forms i.e., the ecdysteroids and the vertebrate-type sex steroids 415 (Subramoniam 2017b). Distinctly, the difference in the roles of these two main steroids is ecdysteroid controls moulting process and gonadotrophic hormones and vertebrate-type sex hormones have main roles in stimulating vitellogenesis (Subramoniam 2011). 420

The results of our present study showed that concentrations of androst and estran fluctuated with sexes and stages of gonad maturity. These new steroid hormones have positive roles in gonadal maturation of spiny lobster. Androst was abundantly found in male 425 spiny lobster and estran expression was much more frequent in female spiny lobster. Moreover, estran was detected in male spiny lobster and androst was also detected in female spiny lobster. Both hormones were

Table 3. Identified fatty acids concentration (%) during gonadal maturation of female spiny lobster (Panulirus homarus).

Whole eventsk ableton dung period validgenesis (1) JJJ mounty Condect ond ad CPS of the Addition of a condective ad CPS of the Addition of a condective ad CPS of the Addition of a condective ad CPS of the Addition of add	^{CIN}	(chamba of campa)	Dody moight (a)	Mativity.	Idontified fatty acide	(70)
Whour everals ablation during primary vitellogenesis (1) 128 Immatue 21:3:10-Gradectorid actionmethy ferter 22:3:10-Gradectorid actionmethy ferter Whour eyerals ablation during secondary vitellogenesis (1) 13:3 Immatue 21:3:10-Gradectorid actionmethy ferter 22:3:10-Gradectorid actionmethy ferter Whour eyerals ablation during secondary vitellogenesis (1) 13:3 Immature 21:3:10-Gradectorid actionmethy ferter 22:3:10-Gradectorid actionmethaning end vectorid Whour eyerals ablation during secondary vitellogenesis (1) 13:3 Immature 20:3:10-Gradectorid actionmethaning end vectorid 2 Whour eyerals ablation during secondary vitellogenesis (1) 13:3 Immature 20:3:0:Gradectorid actionmethaning end vectorid 2 Whour eyerals ablation during primary vitellogenesis (1) 13:3 Immature 20:3:0:Gradectorid actionmethaning end vectorid 2 Whour eyerals ablation during primary vitellogenesis (1) 13:3 Immature 20:3:0:Gradectorid actionmethaning end vectorid 2 Whour eyerals ablation during maturation (1) 13:3 13:3:0:Gradectorid actionmethaning end vectorid 2 Whour eyerals ablation during maturation (1) 13:3:0:Gradectorid actionmethaning end vectorid 2 2 Whour eyerals ablation during maturation (1)			bouy weight (g)	ואומרחוורא	ומבוותוובת ומרוא מרומא	(02)
Whour eyestik ablation during secondary viellogeneis (1) 15 Immature CVED propresentancia add, sing team ergy Action endors with ergenties add, sing team ergy Action endors with ergenties add, sing team ergy Action endors and add add add add add add add add add	-	Without eyestalk ablation during primary vitellogenesis (1)	128	lmmature	Octadecanoid acid/Stearic acid	22.32 ± 0.00
Without eyeatik ablation during secondary vitellogenesis (1) 136 Immature Prepension add, mitry 3 dimension ethy ease secondary vitellogenesis (1) 13 Prepension add, mitry 3 dimension ethy ease secondary vitellogenesis (1) 13 Immature Prepension add, mitry 5 dimension ethy ease secondary vitellogenesis (1) 13 Immature Prepension add, mitry 5 dimension ethy ease secondary vitellogenesis (1) 13 Immature Prepension add, mitry ease secondary vitellogenesis (1) 13 Immature Prepension add, mitry ease secondary vitellogenesis (1) 13 Immature Preprenoid add, mitry ease secondary vitellogenesis (1) 13 13 13 14 14 14 14 14 14 14 14 14					9,12,15-Octadecatrienoic acid,methyl ester	2.60 ± 0.00
Whour operade ablation during secondary vielogenesis (1) 136 Immature 2. Persecond add/dx/01.14-turnentlyh, antily seter 2 Whour operade ablation during maturation (1) 134 Enclose condition during maturation (1) 134 Enclose condition during secondary vielogenesis (1) 13 Enclose condition during maturation (1) 14 Enclose condition during maturation (1) 14 Enclose condition during maturation (1) 15 Enclose condition during maturation (1) 15 Enclose condition during maturation (1) 15 15 Enclose condition during maturation (1) 15 15 Enclose condition during maturation (1) 15					Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	2.59 ± 0.00
Whour oystack ablation during secondary viellogenets (1) 136 Immune Periodecronid add/orition during manuation (1) 134 Immune Consider and add/orition during manuation (1) 134 Immune Providecronid add/orition add					2-Prepenoic acid.2-methyl.2-(dimethylamino) ethyl ester	1.15 ± 0.00
Without eyestalk ablation during secondary viellogeneis (1) 126 Immature 9:0:chaderenoid add/Serve add Without eyestalk ablation during maturation (1) 131 Immature 9:0:chaderenoid add/Serve add Without eyestalk ablation during primary viellogeneis (1) 132 Immature 9:0:chaderenoid add/Serve add Other eyestalk ablation during maturation (1) 133 Immature 9:0:chaderenoid add/Serve add Without eyestalk ablation during primary viellogeneis (1) 133 Immature 9:0:chaderenoid add/Serve add Without eyestalk ablation during primary viellogeneis (1) 139 Immature 9:0:chaderenoid add/Serve add Without eyestalk ablation during primary viellogeneis (1) 139 Immature 9:0:chaderenoid add/Serve add Without eyestalk ablation during primary viellogeneis (1) 139 Immature 9:0:chaderenoid add/Serve add Without eyestalk ablation during primary viellogeneis (1) 139 Mature 9:0:chaderenoid add/Serve add Without eyestalk ablation during maturation (1) 139 Mature 9:0:chaderenoid add/Serve add Without eyestalk ablation during maturation (1) 139 0:chaderenoid add/Serve add 0:chaderenoid add/Serve add Without eyestalk ablation during maturation (1) <td< td=""><td></td><td></td><td></td><td></td><td>Pentaderannid arid 4 6 10 14-tetramethyl- methy ester</td><td>0.80 + 0.00</td></td<>					Pentaderannid arid 4 6 10 14-tetramethyl- methy ester	0.80 + 0.00
Throw speak abation during maturation (1) 19 Immoust operank abation during maturation (1) 19 Immoust operank abation during maturation (1) Without operank abation during maturation (1) 19 Immoust operank abation during grimmay vitellogenesis (1) 19 Immoust operank abation during maturation (1) 10 10 Immoust operank abation during primary vitellogenesis (1) 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 10 <td< td=""><td>ç</td><td>Without avortally ablation during corondary uitallocomorie (1)</td><td>301</td><td>mmaturo</td><td>0 Ortadoronoid arid/Oloir arid</td><td>75 06 ± 121</td></td<>	ç	Without avortally ablation during corondary uitallocomorie (1)	301	mmaturo	0 Ortadoronoid arid/Oloir arid	75 06 ± 121
Without ejectalk ablation during maturation (1) 34 Immute Consideration action a	V	עונווטטנ באבאמוע מטומנוטו טעוווט אברטווטמוע עונבווטאבוובאא (ו)	071	IIIIIIIIIII		10.4 1 06.02
Whoker everalk ablation during maturation (1) 13 Immate Discretional action acti					Octadecanoid acid/Stearic acid	16.41 ± 0.00
Wrhout eystalk ablation during maturation (1) 13 Immature Considerend add/Oper edid One eystalk ablation during secondary vitellogenesis (1) 12 Immature Pocoasterend add/Oper edid One eystalk ablation during maturation (1) 13 Immature Pocoasterend add/Oper edid One eystalk ablation during maturation (1) 13 Immature Pocoasterend add/Oper edid Without eystalk ablation during maturation (1) 13 Immature Pocoasterend add/Oper edid Without eystalk ablation during maturation (1) 13 Mature Pocoasterend add/Oper edid Without eystalk ablation during maturation (1) 13 Mature Pocoasterend add/Oper edid Without eystalk ablation during maturation (1) 13 Mature Pocoasterendia add/Oper edid Without eystalk ablation during maturation (1) 13 Mature Pocoasterendia add/Oper edid One eystalk ablation during maturation (1) 13 Mature Pocoasterendia add/Oper edid One eystalk ablation during maturation (1) 13 Mature Pocoasterendia add/Oper edid One eystalk ablation during maturation (1) 13 Mature Pocoasterendia add/Oper edid One eystalk ablation during maturation (1)					Nonadecanoid acid, ethyl ester	1.15 ± 0.00
Without eystalk ablation during maturation (1) 134 Immature broaderendia add/Obits add One eystalk ablation during maturation (1) 123 Immature 130 Control excluding eters (1) 20 catalerendia add/Obits add One eystalk ablation during maturation (1) 130 Immature 130 Control excluding eters (1) 20 catalerendia add/Obits add One eystalk ablation during maturation (1) 130 Immature 130 Contactering add/Obits add 20 catalerendia add/Obits add Without eystalk ablation during maturation (1) 130 Immature 20 catalerendia add/Obits add 20 catalerendia add/Obits add Without eystalk ablation during maturation (1) 130 Immature 20 catalerendia add/Obits add 20 catalerendia add/Obits add Without eystalk ablation during maturation (1) 130 Immature 20 catalerendia add/Obits add 20 catalerendia add/Obits add Without eystalk ablation during maturation (1) 132 Mature 20 catalerendia add/Obits add 20 catalerendia add/Obits add Without eystalk ablation during maturation (1) 132 Mature 20 catalerendia add/Obits add 20 catalerendia add/Obits add Without eystalk ablation during maturation (1) 132 Mature 20 catalerendia add/Obits add 20 catalerendia add/Obits add One eystalk abla					Cyclopentaneundecanoid acid	0.59 ± 0.00
One eyeralki abition during primay vitellogenesis (1) 32 immature occaraterior acid/optic acid One eyeralki abition during maturation (1) 13 immature occaraterior acid/optic acid One eyeralki abition during maturation (1) 13 immature occaraterior acid/optic acid One eyeralki abition during maturation (1) 13 immature occaraterior acid/optic acid Without eyeralki abition during maturation (1) 13 immature occaraterior acid/optic acid Without eyeralki abition during maturation (1) 13 immature occaraterior acid/optic acid Without eyeralki abition during maturation (1) 13 immature occaraterior acid/optic acid Without eyeralki abition during maturation (1) 13 Mature occaraterior acid/optic acid Without eyeralki abition during maturation (1) 13 Mature occaraterior acid/optic acid Without eyeralki abition during maturation (1) 13 Mature occaraterior acid/optic acid Without eyeralki abition during maturation (1) 13 0.00 occaraterior acid/optic acid Without eyeralki abition during secondary vitellogenesis (1) 13 0.00 occaraterior acid/optic acid Without eyeralki abition during secondary vitellogenesis (1) 13 0.00 0.00 One eyeralki abition duri	ć	Without evestalk ablation during maturation (1)	134	Immature	Hexadecanoic acid/Palmitic acid	11.33 + 0.00
One operatik ablation during primary virellogenesis (1) 12 Immature 17-Obscience add/Samily early One operatik ablation during primary virellogenesis (1) 130 Immature 2-Obscience add/Samily early One operatik ablation during primary virellogenesis (1) 130 Immature 2-Obscience add/Samily early Without eyestalk ablation during primary virellogenesis (1) 130 Immature 2-Obscience add/Samily early Without eyestalk ablation during primary virellogenesis (1) 148 Mature 2-Obscience add/Samily early Without eyestalk ablation during primary virellogenesis (1) 130 Immature 2-Obscience add/Samily early Without eyestalk ablation during primary virellogenesis (1) 132 Mature 2-Obscience add/Samily early One eyestalk ablation during primary virellogenesis (1) 132 Mature 2-Obscience add/Samily early One eyestalk ablation during primary virellogenesis (1) 132 Mature 2-Obscience add/Samily early One eyestalk ablation during primary virellogenesis (1) 132 Mature 2-Obscience add/Samily early One eyestalk ablation during primary virellogenesis (1) 132 Mature 2-Opscience add/Samily early One eyestalk ablation during primary virellogenesis (1) 132 Mature 2-Opscience add/Samily early One eyestalk ablation during primary virellogen	9			5	9-Ortaderenoid acid/Oleic acid	407 + 000
One eystak ablation during secondary vitellogenesis (1) 12 immature becate end act/cap/k acid One eystak ablation during secondary vitellogenesis (1) 13 immature becate end act/cap/k acid One eystak ablation during primary vitellogenesis (1) 13 immature becate end act/cap/k acid Without eystak ablation during primary vitellogenesis (1) 13 immature becate end act/cap/k acid Without eystak ablation during primary vitellogenesis (1) 13 Mature becate end act/cap/k acid Without eystak ablation during maturation (1) 13 Mature becate end act/cap/k acid One eystak ablation during maturation (1) 13 Mature processond act/cap/k acid One eystak ablation during maturation (1) 13 Mature processond act/cap/k acid One eystak ablation during primary vitellogenesis (1) 13 Mature processond act/cap/k acid One eystak ablation during primary vitellogenesis (1) 13 Mature processond act/cap/k acid One eystak ablation during primary vitellogenesis (1) 13 Mature processond act/cap/k acid One eystak ablation during primary vitellogenesis (1) 13 Mature processond act/cap/k acid One eystak ablation during primary vitellogenesis (1) 13 Mature processond act/cap/k acid One eystak ablation during primary vitellogenesis (1) 13 Mature processond act/cap/k acid One eystak ablation durin	,	Oue acception during animal action of 11	CC1		12 December and mathed acts	
One eyestak ablation during maturation (1) 12 immature evended endordering actuation endordering maturation (1) 13 immature evended endordering actuation endordering maturation (1) 13 immature evended endordering actuation endordering actuation during primary vitellogenesis (1) 13 immature evended endordering actuation endordering endorderi	t 1		122			4.09 ± 0.00
One eyestalk ablation during maturation (1) 130 Immature 9.Considerential action action Without eyestalk ablation during maturation (1) 148 Mature 9.Considerential action action Without eyestalk ablation during primary vitellogenesis (1) 148 Mature 9.Considerential action action Without eyestalk ablation during primary vitellogenesis (1) 123 Mature 9.Considerential action action Without eyestalk ablation during maturation (1) 131 Mature 9.Considerential action One eyestalk ablation during maturation (1) 131 Mature 9.Considerential action One eyestalk ablation during maturation (1) 131 Mature 9.Considerential action One eyestalk ablation during primary vitellogenesis (1) 131 Mature 9.Considerential action One eyestalk ablation during primary vitellogenesis (1) 131 Decremon action 0.Coloperation action One eyestalk ablation during primary vitellogenesis (1) 131 Decremon action 0.Coloperation action One eyestalk ablation during primary vitellogenesis (1) 131 Decremon action 0.Coloperation action One eyestalk ablation during primary vitellogenesis (1) 131 Decremon action 0.Coloperation action One eyestalk ablation during secondary vitellogenesis (1) 130 Decremon action 0.	ç	One eyestalk ablation during secondary vitellogenesis (1)	6 21	Immature	Uctanoid acid/Caprylic acid	24.08 ± 0.00
One eyestak ablation during maturation (1) 130 Immature sevance acid Without eyestak ablation during primary vitellogenesis (1) 148 Mature 2 Undecennois acid? 2 Undecennois acid? Periodicanid acid? Periodicanid acid? Periodicanid acid? 2 Mature 2 Prependix acid? 2 Mature 2 P					9-Octadecenoid acid/Oleic acid	10.90 ± 0.85
One eyestalk ablation during maturation (1) 130 Immatue 20 catescensic add/2 mitur, add Without eyestalk ablation during primary vitellogenesis (1) 148 Mature 2. Jundecensic add/2 mitur, add Without eyestalk ablation during primary vitellogenesis (1) 148 Mature 2. Jundecensic add/2 mitur, add Without eyestalk ablation during maturation (1) 123 Mature 2. Jundecensic add/2 mitury ster One eyestalk ablation during maturation (1) 121 Mature 2. Stepsonic add/2 mitury ster One eyestalk ablation during maturation (1) 131 Mature 2. Stepsonic add/2 mitury ster One eyestalk ablation during maturation (1) 131 Mature 2. Stepsonic add/2 mitury ster One eyestalk ablation during primary vitellogenesis (1) 131 Mature 2. Stepsonic add/2 mitury ster One eyestalk ablation during secondary vitellogenesis (1) 132 Mature 2. Stepsonic add/2 mitury ster One eyestalk ablation during secondary vitellogenesis (1) 132 Mature 2. Stepsonic add/2 mitury/2 mitury ster One eyestalk ablation during secondary vitellogenesis (1) 135 Mature 2. Stepsonic add/2 mitury/2 mi					Hexanoic acid	1.34 ± 0.00
Without eyestalk ablation during primary vitellogenesis (1) 148 Mature Eudodecanoic acid/amitrit acid Without eyestalk ablation during primary vitellogenesis (1) 122 Mature 2-Preparior acid/3-metryly-3-dimetryly-amittate Without eyestalk ablation during maturation (1) 123 Mature 2-Preparior acid/3-metryly-3-dimetryly-amittate Without eyestalk ablation during maturation (1) 131 Mature 2-Preparior acid/3-metryly-2-dimetryly-amittate One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2-Preparior acid/3-metryly-2-dimetryly-ster One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2-Preparior acid/3-metryly-2-dimetryly-ster One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2-Preparior acid/3-metryly-2-dimetryly-metry One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2-Preparior acid/3-metryly-2-dimetryly-metry One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2-Preparior acid/3-metryly-2-dimetryly-metry One eyestalk ablation during secondary vitellogenesis (1) 151 Mature 2-Preparior acid/3-metryly-ster One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2-Preparior acid/	9	One evestalk ablation during maturation (1)	130	Immature	9-Octadecenoid acid/Oleic acid	27.08 ± 8.74
Without eyestalk ablation during primary vitellogenesis (1) 18 Mature Decadecandia actioNic actioPamitic acid Without eyestalk ablation during secondary vitellogenesis (1) 123 Mature Decadecandia actioNic acid Without eyestalk ablation during maturation (1) 131 Decadecandia actioNic acid Statements/Amethyl ester Without eyestalk ablation during maturation (1) 131 Mature Decadecandia actioNic acid One eyestalk ablation during maturation (1) 131 Mature Decadecandia actioNic acid One eyestalk ablation during primary vitellogenesis (1) 131 Docadecandia actioNic acid One eyestalk ablation during primary vitellogenesis (1) 131 Mature Mature One eyestalk ablation during primary vitellogenesis (1) 132 Mature Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Mature Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Mature Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Oradecandia acid/Stearc Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Mature Prependic acid/Stearc Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Mature Mature Prependic acid/Stearc					2-I Inderennic acid	111 + 0.00
Mithout eyestalk ablation during secondary vitellogenesis (1) 132 Mature 2-Prependic acid/ 2-Prependic acid/ 2-Prependic acid/2-methyl/3-dimethylaminol ethyl ester 2-Prependic acid/3-methyl/3-dimethylaminol ethyl ester 3-11.4-Eurosatenoic acid/3-methyl/3-dimethylminol ethyl ester 3-1	7	Without evectalk ablation during primary vitallogenesis (1)	148	Maturo	E onacconoic acid/Dalmitic acid	578 ± 0.00
Without eyestalk ablation during secondary vitellogenesis (1) 122 Mature Procreaterion dod, ethyl ester Without eyestalk ablation during maturation (1) 123 Mature Concreaterion dod, ethyl ester Without eyestalk ablation during maturation (1) 131 Mature Concreaterion dod, ethyl ester One eyestalk ablation during maturation (1) 131 Mature Mature Concreaterion dod, ethyl ester One eyestalk ablation during primary vitellogenesis (1) 131 Mature Mature Mature One eyestalk ablation during primary vitellogenesis (1) 131 Mature Mature Mature One eyestalk ablation during primary vitellogenesis (1) 132 Mature Mature Mature One eyestalk ablation during primary vitellogenesis (1) 132 Mature Mature Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Mature Mature Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Mature Mature Mature One eyestalk ablation during secondary vitellogenesis (1) 132 Mature Mature Mature One eyestalk ablation Mature 132 Mature Mature Mature One eyestalk ablation Mature 130 Mature <td< td=""><td></td><td>minious chestains assantion daming primary michogenesis (1)</td><td></td><td></td><td></td><td></td></td<>		minious chestains assantion daming primary michogenesis (1)				
Without eyestalk ablation during secondary vitellogenesis (1) 152 Mature 2Prependix add.Thertkyl patintate Without eyestalk ablation during secondary vitellogenesis (1) 151 Mature 2Prependix add.Thertkyl ester Without eyestalk ablation during maturation (1) 151 Mature 2Prependix add.Thertkyl ester Without eyestalk ablation during maturation (1) 151 Mature 2Prependix add.Thertkyl ester One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2Prependix add.Thertkyl ester One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2Prependix add.Thertkyl ester One eyestalk ablation during primary vitellogenesis (1) 151 Mature 2Prependix add.Thertkyl ester One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2Prependix add.Thertkyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2Prependix add.Thertkyl address One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2Prependix add.Thertkyl address One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2Prependix add.Phileter One eyestalk ablation during secondary vitellogenesis (1) 15					9-Octadecenold acia/Oleic acia	4.98 ± 0.00
Without eyestalk ablation during secondary vitellogenesis (1) 152 Mature 2.Perpendix add.admethyl ester Without eyestalk ablation during maturation (1) 151 Mature 2.Perpendix add.admethyl ester Without eyestalk ablation during maturation (1) 151 Mature 2.Perpendix add.admetryl ester Without eyestalk ablation during maturation (1) 151 Mature 2.Perpendix add.admetryl ester One eyestalk ablation during primary vitellogenesis (1) 151 Mature 0.cradecanoid acid?Stentix add One eyestalk ablation during secondary vitellogenesis (1) 151 Mature 0.cradecanoid acid?Greenic add One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2.Perpenoix cadd.amethyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2.Perpenoix cadd.amethyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2.Perpenoix cadd.amethyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2.Perpenoix cadd.amethyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2.Perpenoix cadd.amethyl ester One eyestalk ablation 0.ne 2.Perpenoix cadd.					Hexadecanoid acid, ethyl ester/Ethyl palmitate	3.32 ± 0.00
Without eyestalk ablation during secondary vitellogenesis (1) 132 Mature Cycloperanemicaenoid acid Without eyestalk ablation during maturation (1) 151 Mature Cycloperanemicaenoid acid Without eyestalk ablation during maturation (1) 151 Mature Cycloperanemicaenoid acid One eyestalk ablation during primary vitellogenesis (1) 151 Mature Cycloperanemicaenoid acid One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-Preprint acid One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-Preprint acid One eyestalk ablation during primary vitellogenesis (1) 150 Mature 2-Preprint acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2-Preprint acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2-Preprint acid Previout acid/2-metryl-metryly ester One eyestalk ablation during maturation (1) 150 Mature 2-Preprint acid/2-metryl-metryly ester Previout acid/2-metryl-metryl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Cycloperanemicaenoli acid/2-metryl-metryl ester One eyestalk ablation One eyestalk a					2-Prepenoic acid.2-methyl.2-(dimethylamino) ethyl ester	0.07 ± 0.00
Without eyestalk ablation during maturation (1) 151 Mature 0.515 Condectange acid 0.515 Condectange acid 0.512 Condectange acid 0.	¢	Without evectally ablation during carondary vitallogenesis (1)	152	Mature	Cvclonentaneunderannid acid	+
Without eyestalk ablation during maturation (1) 151 Mature Occaseroiotic acid, methyl ester Victiont eyestalk ablation during maturation (1) 151 Mature Coccaseroiotic acid, methyl ester One eyestalk ablation during primary vitellogenesis (1) 151 Mature Coccaseroiotic acid, methyl ester One eyestalk ablation during primary vitellogenesis (1) 152 Mature Coccaseroiotic acid, methyl ester One eyestalk ablation during primary vitellogenesis (1) 152 Mature Coccaseroiotic acid, methyl ester One eyestalk ablation during secondary vitellogenesis (1) 152 Mature Coccaseroiotic acid, methyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Soccaseroiotic acid, methyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Soccaseroiotic acid, methyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Soccaseroiotic acid, methyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Soccaseroiotic acid, methyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Soccaseroic acid/Palmitic acid One eyestalk ablation One e	5	minou cyconain abianon aging secondary michogenesis (1)	201		October manean acta	000 + 200
912:15-Octadecantenio: acid, methyl ester. 912:15-Octadecantenio: acid, methyl ester. Without eyestalk ablation during maturation (1) 151 Mature 9.0.2.1.5-Octadecantenio: acid, anethyl ester. Octadecanol 32.0.0000 9.0.0.1.1.5-Octadecantenio: acid, anethyl ester. 32.0.00000 One eyestalk ablation during primary vitellogenesis (1) 151 Mature 0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.					Uctadecanoid acid/Stearic acid	9.03 ± 0.00
Without eyestalk ablation during maturation (1) 151 Mature 2-Propendic acid, methyl acter Properoid 3-boossensic acid, methyl acter 3-boossensic acid, methyl acter One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-propendic acid, methyl acter One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-propendic acid, methyl acter One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-propendic acid, methyl acter One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-propendic acid, methyl acter One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2-propendic acid, methyl acter One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2-propendic acid, methyl acter One eyestalk ablation during maturation (1) 150 Mature 2-propendic acid, methyl acter One eyestalk ablation during maturation (1) 150 Mature 2-propendic acid, methyl acter One eyestalk ablation during maturation (1) 150 Mature 2-propendic acid, methyl acter One eyestalk ablation during maturation (1) 169 11.4-Eticostricorid durineryl acter 2-propendic acid,					9,12,15-Octadecatrienoic acid,methyl ester	4.50 ± 0.53
Trependic acid2-methyl2-(dimethylamino) eityl ester The constraint of the primary vitelogenesis (1) 151 Mature 2-Prependic acid2-methyl2-(dimethylamino).butyl ester One eyestalk ablation during primary vitelogenesis (1) 152 Mature 2-Prependic acid2-methyl2-(dimethylamino).butyl ester One eyestalk ablation during primary vitelogenesis (1) 152 Mature 2-Prependic acid2-methyl2-(dimethylamino).butyl ester One eyestalk ablation during primary vitelogenesis (1) 152 Mature 2-Prependic acid2-methyl2-(dimethylamino).butyl ester One eyestalk ablation during secondary vitelogenesis (1) 150 Mature 2-Prependic acid2-methyl2-(dimethylamino) ethyl ester One eyestalk ablation during secondary vitelogenesis (1) 150 Mature 2-Prependic acid2-methyl2-dimethylamino) ethyl ester One eyestalk ablation during maturation (1) 150 Mature 2-Createcenoid acid.Oneic acid2-methyl2-methyl ester One eyestalk ablation during maturation (1) 10-Dicatecenoid acid.Prependic acid.Prepend					Cyclopropanepentanoic acid, 2-undercyl-, methyl ester, trans	2.09 ± 0.47
Without eyestalk ablation during maturation (1) 151 Mature 13-Discosencia caid, methyl ester Octadesenold 9-Octadesenold acid/Oleic caid Postal Postal Postal Doe eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-Prependic acid/Palmitic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadesenoid acid Postalesenoid acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadesenoid acid Postalesenoic acid					2-Prepenoic acid.2-methyl.2-(dimethylamino) ethyl ester	1.32 ± 0.00
Without eyestalk ablation during maturation (1) 151 Mature Occadecenoid acid/Stearic acid Butanoic acid/Stearic acid Butanoic acid/Stearic acid Butanoic acid/Stearic acid Butanoic acid/Stearic acid Butanoic acid/Stearic acid Cyclopropare pentanoic acid/Stearic acid Cyclopropare pentanoic acid/Stearic acid Cyclopropare pentanoic acid/Stearic acid Cyclopropare pentanoic acid/Stearic acid String acid/Stearic acid Cyclopropare pentanoic acid/Stearic acid String Hexadecanoid acid/Stearic acid Cyclopropare pentanoic acid/Stearic acid String acid/Stearic acid String acid/Stearic acid Butanoic acid/Stearic acid String acid/Stearic acid String acid String acid/Stearic acid String acid String acid/Stearic acid String acid/Stearic acid String acid String acid/Stearic acid String acid String acid/Stearic acid String acid Strin					13-Docosennic acid methyl ester	0.79 + 0.00
writout tystak anaton during primary vitellogenesis (1) D1 watue Occardetendia acid/Oleic acid One eyestalk ablation during primary vitellogenesis (1) 152 Mature Perpenoid acid/Oleic acid One eyestalk ablation during primary vitellogenesis (1) 152 Mature Prodetendia acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 152 Mature Progenoid acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Prodetenoid acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Prodetenoid acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Prodetenoid acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Prodetenoid acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Prodeteenoid acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Prodeteenoid acid/Oleic acid One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Prodeteenoid acid/Oleic acid One eyestalk ablation 0 Evencio acid/Oleic acid Evencio acid/Oleic acid Evencio One eyestalk ablation 0 Evencio acid/Oleic aci	c	الالتطفينية ومنقطها والمتراجع والمنشوط والمتعاولات المتحدد المنافعات المرابع	151	NA - 4		
One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-Prepanoic acid/2 (friftluoroactty)lamino)-burdy ester One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-Prepanoic acid/2 (friftluoroactty)lamino)-burdy ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/DeitetyJamino) One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/DeitetyJamino One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/DeitetyJamitic acid One eyestalk ablation during maturation (1) 150 Mature 9-Octadecenoid acid/DeitetyJamitic acid One eyestalk ablation during maturation (1) 149 Mature 9-Octadecenoic acid/3-methyl.ester Prependic acid/3-methyl ester 0-10-ctadecenoic acid/3-methyl.ester 0-10-ctadecenoic acid/3-methyl.ester One eyestalk ablation 11,14-methyl.ester 0-10-ctadecenoic acid/3-methyl.ester One eyestalk ablation 11,14-methyl.ester 0-10-ctadecenoic acid/3-methyl.ester One eyestalk ablation 11,14-methyl.ester 0-10-ctadecenoic acid/3-methyl.ester One eyestalk ablation 149 Mature 0-ttadecenoic acid/3-methyl.ester	ע	without eyestalk adjation quring maturation (1)	10	Mature	Uctagecanolg acig/stearic acig	10.28 ± 0.00
One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-Prepenotic acid/2-methyl/amino), butyl ester One eyestalk ablation during secondary vitellogenesis (1) 152 Mature 2-Prepenotic acid/2-methyl/amino) ethyl ester One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 2-Prepenotic acid/2-methyl/amino) ethyl ester 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-otcadesenoid acid 1 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-otcadesenoid acid/2-methyl ester 1 1 One eyestalk ablation 0ne eyestalk ablation 1.1.1.4-Eicosatrienoic acid/Palmitic acid 1 1 One eyestalk ablation 149 Mature 1.1.4-Eicosatrienoic acid/Palmitic acid 1 1 One eyestalk ablation 10 0.0					9-Octadecenoid acid/Oleic acid	12.35 ± 0.00
One eyestalk ablation during primary vitellogenesis (1) 152 Mature 2-Prepenoic acid/Palmitic acid 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Hexadecanoic acid/Palmitic acid 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/Palmitic acid 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/Palmitic acid 1 One eyestalk ablation 11.0-Eicossatrenoic acid/Palmitic acid 1 1 One eyestalk ablation 811.1.4-Eicossatrenoic acid/Palmitic acid 1 1 One eyestalk ablation 11.0-Octadecenoid acid/Palmitic acid 1 1 One eyestalk ablation 1.14-Eicossatrenoic acid/Palmitic acid 1 1 One eyestalk ablation 1.10 2-Prepenoic acid/Palmitic acid 1 1 One eyestalk ablation 1 2-Prepe					Butanoic acid,2((trifluoroacthyl)amino)-,butyl ester	1.53 ± 0.00
One eyestalk ablation during primary vitellogenesis (1) 152 Mature Hexadecanoic acid/Palmitic acid 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Hexadecanoic acid/Palmitic acid 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octopropane perfanoic acid/Palmitic acid 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octopropane perfanoic acid/Palmitic acid 1 One eyestalk ablation 141/14-Eicosatrienoic acid/Palmitic acid 1 1 One eyestalk ablation 311/14-Eicosatrienoic acid, methyl ester 1 1 One eyestalk ablation 149 Mature 1 1 1 One eyestalk ablation 149 Mature 69.12-methyl-ster 1 1 One eyestalk ablation 1 1 9 0-ctadecanoic acid, methyl ester 1 1 One eyestalk ablation 1 1 1 1 1 1 1 1 1 One eyestalk ablation 1 1 1 1 1 1 1 1 1 1 <t< td=""><td></td><td></td><td></td><td></td><td>2-Prepenoic acid.2-methyl.2-(dimethylamino) ethyl ester</td><td>0.44 ± 0.00</td></t<>					2-Prepenoic acid.2-methyl.2-(dimethylamino) ethyl ester	0.44 ± 0.00
One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-octadecanoid acid/Palmitic acid 9-octadecanoid acid/Palmitic acid 9-octadecanoid acid/Palmitic acid 9-octadecanoid acid/Palmitic acid 11.4.Eicosatienoic acid, methyl ester 7.1.1.4.Eicosatienoic acid, methyl ester 11.0.ctadecanoid acid/Palmitic acid 9-octadecanoid acid, methyl ester 9-octadecanoid acid, 2-methyl ester 9-octadecanoid acid, 2-methyl ester 9-octadecanoid acid, 2-methyl ester 9-octadecanoid acid, 2-methyl ester 7-0-octadecanoid acid, 2-methyl ester	10	One evertally ablation during mrimany vitallogenesis (1)	157	Matura	Havaderanoir arid/Dalmitir arid	1555 + 0.00
One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/Oleic acid 1 One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/Oleic acid 1 One eyestalk ablation during maturation (1) 150 Mature 9-Octadecenoid acid/Palmitic acid 1 One eyestalk ablation during maturation (1) 149 Mature 1-Octadecenoid acid/Palmitic acid 1 One eyestalk ablation during maturation (1) 0-Octadecenoid acid/Palmitic acid 1 1 One eyestalk ablation during maturation (1) 149 Mature 2-Prepenoic acid/Palmitic acid 1 One eyestalk ablation during maturation (1) 0-Octadecanoic acid/Palmitic acid 1 1 One eyestalk ablation during maturation (1) 0-Octadecanoic acid/Palmitic acid 1 One eyestalk ablation 149 Mature 2-Prepenoic acid/Palmitic acid 1 One eyestalk ablation 1 0-Octadecanoic acid/Palmitic acid 1 1 One eyestalk ablation 11-Octadecanoic acid/Palmitic acid 1 1 1 1 One eyestalk ablation 10 0-Octadecanoic acid/Palmitic acid 1	2		201	ואומרמוב	riekauekanibik akiu/r aninink akiu Andonontanonindoranoid arid	000 + cc.cl
One eyestalk ablation during secondary vitellogenesis (1) 150 Mature Undecanoic acid/Oleic acid 1 Cyclopropane pentancic acid/antic acid 1 1 1 1 Readecanoic acid/antic acid 1 1 1 1 Readecanoic acid/antic acid 1 1 1 1 Readecanoic acid/antic acid 1<						
One eyestalk ablation during secondary vitellogenesis (1) 150 Mature 9-Octadecenoid acid/Oleic acid 1 Revadecanoic acid/Palmitic acid 8.11,14-Eicosatrienoic acid, methyl ester 1 Revadecanoic acid, methyl ester 9.11,14-Eicosatrienoic acid, methyl ester 1 Revadecanoic acid, methyl ester 9.11,0ctadecanoic acid, methyl ester 1 Revadecanoic acid, methyl ester 1.10,0ctadecanoic acid, methyl ester 1 One eyestalk ablation 1.10,0ctadecanoic acid, methyl ester 1 Auring maturation (1) 2-Prepenoic acid, 14-methyl-inethyl ester 1 Octadecanoic acid, 14-methyl-inethyl ester 1 1 One eyestalk ablation 1.10,0ctadecanoic acid, 14-methyl ester 1 One eyestalk ablation 1.10,0ctadecanoic acid, methyl ester 1 One eyestalk ablation 1.10,0ctadecanoic acid, 14-methyl ester 1 One eyestalk ablation 0.12-Octadecanoic acid, methyl ester 1 One eyestalk ablation 1 0.0ctadecanoic acid, 1-methyl ester 1 One eyestalk ablation 1 0.0ctadecanoic acid, 1-methyl ester 1 One eyestalk ablation 1 0.0ctadecanoic acid, 1-methyl ester 1					Cyclopropane pentanoic acid, 2-undercyl-, methyl ester, trans	5.40 ± 0.61
1 Hexadecanoic acid/Palmitic acid 1 8,11,14-Eicosatrienoic acid, methyl ester 8,11,14-Eicosatrienoic acid, methyl ester 1 0ne eyestalk ablation 8,11,14-Eicosatrienoic acid, methyl ester 1 0ne eyestalk ablation 149 Mature 2-Prepenoic acid, methyl ester 1 0ne eyestalk ablation 149 Mature 2-Prepenoic acid, methyl ester 1 0ne eyestalk ablation 149 Mature 2-Prepenoic acid, methyl ester 1 0 condecenci acid, methyl ester 0 condecenci acid, methyl ester 1 1 0 condecenci acid, methyl ester 0 condecenci acid, methyl ester 1 1 149 Mature Decanic acid, methyl ester 1 1 15,9,12-Octadecatrieonic acid, methyl ester 2-Prepenoic acid, methyl ester 2-Prepenoic acid, methyl ester 2-Prepenoic acid, methyl ester 16,9,12-Octadecanic acid, methyl ester 2-Prepenoic acid, methyl ester 2-Prepenoic acid, methyl ester 2-Prepenoic acid, methyl ester 17,000 0 condecen-12-ynoic acid, methyl ester 2-Prepenoic acid, methyl ester 2-Prepenoic acid, methyl ester 2-Prepenoic acid, methyl ester 16,000 0 condecen-12-ynoic acid, methyl ester	11	One eyestalk ablation during secondary vitellogenesis (1)	150	Mature	9-Octadecenoid acid/Oleic acid	11.39 ± 0.00
Sill, 14-Eicosatrienoic acid, methyl ester Bill, 14-Eicosatrienoic acid, methyl ester Pentadecanoid acid, 14-methyl-imethyl ester 1-Octadecanoic acid, methyl ester 1-Octadecanoic acid, methyl ester 1-Octadecanoic acid, methyl ester 1-Dotadecanoic acid, methyl ester 1-Octadecanoic acid, methyl ester 1-Beranic acid, methyl ester 1-Beranic acid, methyl ester 1-Beranic acid, 1-methylethyl ester 0 <t< td=""><td></td><td></td><td></td><td></td><td>Hexadecanoic acid/Palmitic acid</td><td>10.12 ± 0.00</td></t<>					Hexadecanoic acid/Palmitic acid	10.12 ± 0.00
One eyestalk ablation 149 Mature Periadecanoid acid,14-methyl-,methyl ester 1 One eyestalk ablation 11-Octadecanoic acid,2-methyl,2-(dimethylamino) ethyl ester 1 One eyestalk ablation 149 Mature 1 1 One eyestalk ablation 149 Mature 1 1 1 One eyestalk ablation 149 Mature 1 1 1 Ore eyestalk ablation 10 0 0 0 1 Ore eyestalk ablation 10 0 0 0 1 One eyestalk ablation 149 Mature 1 1 1 1 One eyestalk ablation 0 10 0 0 0 1 Oreadocinci acid, nethyl ester 0 0 0 0 0 Oreadocinci acid, nethyl ester 0 0 0 0 0 Oreadocinci acid, nethyl ester 0 0 0 0 0					8,11,14-Eicosatrienoic acid, methyl ester	4.20 ± 0.00
One eyestalk ablation 11-Octadecanoic acid, methyl ester One eyestalk ablation 149 Mature Hexadecanoic acid, Palmitic acid 1 Our eyestalk ablation 149 Mature 1 One eyestalk ablation 149 Mature 140 1 Our eyestalk ablation 10 0 0 1 1 Our eyestalk ablation 10 0 0 1					Pentadecanoid acid.14-methyl-methyl ester	2.44 ± 0.83
One eyestalk ablation 149 Mature 2-Prepension acid/2-methyl/2-(dimethylamino) ethyl ester 1 One eyestalk ablation 149 Mature 2-Prepension acid/2-methyl/2-(dimethylamino) ethyl ester 1 during maturation (1) Decanoic acid/1-methylethyl ester 0 1 6,9,12-Octadecatrieonic acid, methyl ester 9-Octadecatrieonic acid, methyl ester 1 7-Prepension acid, methyl ester 0 0 0 6,9,12-Octadecatrieonic acid, methyl ester 0 0 0 7-Prepension acid, methyl ester 0 0 0 0 7-Prepension acid, methyl ester 0 0 0 0 0 7-Prepension acid, methyl ester 0 <t< td=""><td></td><td></td><td></td><td></td><td>11. Octadoranoir arid mothyl octar</td><td>162 ± 0.00</td></t<>					11. Octadoranoir arid mothyl octar	162 ± 0.00
One eyestalk ablation 149 Mature 2-trepenot acto_z-menty_iz-tumenty_amino) etnyl ester Ouring maturation (1) Decanoic acid/Palmitic acid 0,912-Octadecanticonic acid, methyl ester 0,912-Octadecanticonic acid, methyl ester 9-Octadecanticonic acid, methyl ester 9-Octadecancic acid, methyl ester 0,912-Octadecancic acid, methyl ester 0,912-Octadecancic acid, methyl ester 9-Octadecancic acid, methyl ester 0,912-Octadecancic acid, methyl ester 0,912-Octadecancic acid, methyl ester 9-Octadecancic acid, methyl ester 0,912-Octadecancic acid, methyl ester 0,912-Octadecancic acid, methyl ester 9-Octadecancic acid, methyl ester 0,912-Octadecancic acid, methyl ester 0,902-octadecancic acid, methyl ester 9-Octadecancic acid, methyl ester						000 I 70.1
Une eyestaix ablation during maturation (1) Decanoic acid, 1-methylethyl ester 6,9,12-Octadecartieonic acid, methyl ester 9-Octadecen-12-ynoic acid, methyl ester 2-Prepenoic acid, methyl ester 9-Octadecanoic acid, methyl ester 7-Colopropanepentanoic acid, 2-undercy1-methyl ester 9-ortadecanoid acid, 14-methyl ester, trans	Ċ				2-Prepenoic acid,2-metnyi,2-(dimetnyiamino) etnyi ester	0.38 ± 0.00
Decanoic acid, 1-methylenyl ester 6,9,12-Octadecarrieonic acid, methyl ester 9-Octadecen-12-ynoic acid, methyl ester 2-Prepenoic acid, methyl ester 9-Octadecanoic acid, methyl ester, trans Pertodecanoid acid 14-methyl- methyl ester, trans	7	Une eyestaik ablation	149	Mature	Hexadecanoic acid/Palmitic acid	$1/.24 \pm 0.00$
er nino)ethyl ester methyl ester, trans		during maturation (1)			Decanoic acid, 1-methylethyl ester	5.33 ± 0.00
nino)ethyl ester methyl ester, trans ster					6,9,12-Octadecatrieonic acid, methyl ester	2.92 ± 0.00
nino)ethyl ester methyl ester, trans star					9-Octadecen-12-ynoic acid, methyl ester	2.06 ± 0.00
lercyl-,methyl ester, trans ethyl ester					2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	1.65 ± 0.28
lercyl-,methyl ester, trans ethyl ester					9-Octadecanoic acid, methyl ester	1.58 ± 0.00
-					Cyclopropanepentanoic acid, 2-undercyl-, methyl ester, trans	1.25 ± 0.00
					Pentadecanoid acid,14-methyl-, methyl ester	0.45 ± 0.00

1	Ireatments				
No	(number of sample)	Body weight (g)	Maturity	Identified fatty acids	(%)
13	One eyestalk ablation during primary vitellogenesis after 30 days in captivity (1)	169	Mature	Octadecanoid acid/Stearic acid	17.78 ± 0.00
				9-Octadecenoid acid/Oleic acid	16.74 ± 0.00
				9,12,15-Octadecatrienoic acid, methyl ester	1.69 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	1.33 ± 0.00
				Phosphorothioc acid,O,O-diisopropyl ester, S-ester-N	1.11 ± 0.00
				2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	0.90 ± 0.00
				Octadecanoic acid, phenylmethyl ester	0.67 ± 0.00
14	One eyestalk ablation during secondary vitellogenesis after 30 days in captivity (1)	174	Mature	9-Octadecenoid acid/Oleic acid	18.80 ± 0.00
				Octadecanoid acid/Stearic acid	15.81 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	4.98 ± 1.23
				9,12,15-Octadecatrienoic acid, methyl ester	1.60 ± 0.00
				2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	1.33 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	0.69 ± 0.00
15	One eyestalk ablation during maturation after 30 days in captivity (1)	163	Mature	Octadecanoid acid/Stearic acid	16.45 ± 0.00
				9-Octadecenoid acid/Oleic acid	9.77 ± 0.00
16	Two eyestalk ablation during primary vitellogenesis after 30 days in captivity (1)	172	Mature	Octadecanoid acid/Stearic acid	11.04 ± 0.00
				Cyclopentaneundecanoid acid	8.74 ± 0.00
				Cyclopropanepentanoic acid,2-undercyl-,methyl ester, trans	4.53 ± 0.75
				12,15-Octadecadiynoic acid, methyl ester	2.33 ± 0.00
				2-Prepenoic acid,2-methyl-,2-(dimethylamino)ethyl ester	1.45 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	1.32 ± 0.00
17	Two eyestalk ablation during secondary vitellogenesis after 30 days in captivity (1)	170	Mature	Octadecanoid acid/Stearic acid	14.39 ± 0.00
				9-Octadecenoid acid/Oleic acid	13.01 ± 0.00
				Pentadecanoid acid,14-methyl-, methyl ester	2.86 ± 0.00
18	Two eyestalk ablation during maturation after 30 days in captivity (1)	168	Mature	Octadecanoid acid/Stearic acid	11.95 ± 0.00
				9-Octadecenoid acid/Oleic acid	11.33 ± 0.00

430 detected in low concentrations and showed their limited roles in stimulating gonadal development in different sexes of spiny lobsters.

435

Injections of thyroxine hormone in male spiny lobster affect androst and estran concentrations. Concentrations of androst in male spiny lobsters with thyroxine injection were lower compared to those without thyroxine injection. Distinctly, estran concentration was higher in male spiny lobster with thyroxine injection compared to those without thyroxine injection.

- These results showed that the roles of androst and 440 estran were related to the thyroxine injection as was indicated by the decreased androst concentration in the male gonads. These facts showed that androst as a stimulator of gonad development in male spiny lob-
- 445 ster was affected by thyroxine hormone. The relation between thyroxine hormone and steroid hormone remain unclear. However, within these results it is shown that thyroxine suppressed steroid hormone synthesis. If this condition continues, faster growth 450 rate caused by thyroxine injection in male spiny lobster is obviously necessary for maturation. In Decapoda,
- thyroxine hormone functions to stimulate somatic growth in Penaeus monodon (Pillai et al. 1987) and Macrobrachium rosenbergii (Roustaian and Gaik 2006).
- 455 Estran has a role in the mature females compared to immature forms. This condition was shown by the concentrations of estran in immature female spiny lobsters even with one eyestalk ablation that were not able to increase compared to the increase in its concentration in
- mature female spiny lobsters. The observation of 460 Quackenbush (1994) supported this result that small female lobsters at the first stage of gonad maturity delayed their reproductions by allowing moulting process to obtain larger sizes. During vitellogenesis stages and treatments, mature female spiny lobsters showed 465 fluctuated concentrations of androst and estran. Yan
- et al. (2017) also showed that steroids concentrations in the gonad during reproductive cycle tended to fluctuate due to biotic factors (maturation, reproductive, food 470 availability and age) and abiotic factors (photoperiodi-
- city, temperature, pH and dissolved oxygen). Since those biotic factors were relatively similar during the experiment, in this study it was assumed that abiotic factors played roles in influencing the level of vitellogenesis that further affected the expressions of androst and estran. 475

Androst and estran were detected in low concentrations in mature female with one eyestalk ablation both during a short-time exposure and a long-time exposure (after 1, month in captivity). In contrast, androst was not

480 found in all vitellogenesis stages in mature female spiny lobsters without eyestalk ablation and those with two eyestalk ablations after one month in captivity. The

practice of eyestalk ablation involves the losing of endogenous moult and vitellogenesis inhibiting hormone (VIH) and lead to the induction of ovarian 485 maturation (Kumar et al. 2018). It is shown that one eyestalk ablation also suppressed androst and estran during vitellogenesis phases. Probably, the availability of vitellogenesis stimulating hormone (VSH) reduced androst and estran into a basal level. These facts need 490 to be clarified in the future study to clarify the relation among androst, estran, VIH and VSH. Quackenbush (1994), Subramoniam and Kirubagaran (2010), and Subramoniam (2011) reported that, VIH and VSH showed inhibitory and stimulatory effects on ovarian 495 growth, vitellogenesis, and yolk regulations of lobsters. In addition, it is also needed to confirm the importance of one eyestalk ablation in mature spiny lobsters. In the present study it was found that female spiny lobsters without eyestalk ablation could produce optimal 500 maturation stage during treatment with high sterol diets. Due to the pressure of animal right it is necessary to support the other option like feeding with high cholesterol diets to replace eyestalk ablation.

In mature female spiny lobsters without eyestalk abla-505 tion and with two eyestalk ablation, estran increased two folds followed by vitellogenesis stages. The peak concentration of estran was reached during secondary vitellogenesis and maturation stage. It is shown that cholesterol from feed is converted into estran by the hepatopancreas 510 and further transported to the ovaries during vitellogenesis similar to those found by Fairs et al. (0, 1990)) in Nephrops norvegicus and Penaeus monodon. Spiny lobster is able to convert exogenous cholesterol obtained from the diet into sex steroids such as progesterone, 17-515 hydroxyprogesterone, androstenedione, testosterone, and moulting hormones such as ecdysterone (Kanazawa and Teshima 1971; Burns et al. 1984).

Fatty acids accumulations that were found during gonadal maturation of male and female spiny lobsters 520 had high variety. Feed such as squid and fish meat that were given to spiny lobster during this study contained a lot of lipid. Metabolism of lipid in crustacean is located in the hepatopancreas as the main site and the products were then accumulated in the muscle and the ovaries 525 (Swevers et al. 1991). Garofalaki et al. (2006) showed that fatty acid contents of the muscle and hepatopancreas of P. vulgaris were low, but phospholipids were available abundantly in these organs. Concentration of lipid in the 530 hepatopancreas was found to decrease as a result of its mobilisation into the gonads during vitellogenesis and increasing lipid accumulation in the ovaries at oogenesis phases (Iromo et al. 2014). These facts suggest that ovarian vitellogenin is synthesised in the hepatopancreas, and later transported into the ovaries (Yan et al. 2017). 535

Fatty acids compounds were also found abundant and showed no single fatty acid that played a dominant role in the gonadal maturation. Stearic acid is the most dominant fatty acid found in mature male spiny lobster on two

- 540 different treatments of thyroxine hormone. This means that injection of thyroxine is not related to the elevation of stearic acid concentration in the gonads of mature male spiny lobsters. Immature female spiny lobsters showed a more complex condition in terms of fatty acids accumu-
- 545 lation. There are stearic acid, oleic acid, and palmitic acid in spiny lobsters without eyestalk ablation. Moreover, only caprylic acid and oleic acid were dominant fatty acids found in spiny lobster with one eyestalk ablation. Fatty acids depositions in the gonad of female spiny lobsters
- 550 with mature gonads had high varieties. Fatty acids that were also found with high varieties were caprylic acid and oleic acid. Mature female spiny lobsters with one eyestalk ablation had different fatty acids patterns where palmitic acid, cyclopentaneundecanoid acid, and oleic acid were
- 555 the most abundant in the ovaries. In addition, for a long time exposure of eyestalk ablation, only two fatty acid classes were found *i.e.*, stearic acid and oleic acid.

This study showed a low variety of nutrients identified in immature female compared to mature female

- 560 spiny lobsters. Physiological changes caused by fatty acids variation are related to hormonal changes in female brood stock (Yano 1998). It was found that fatty acids deposition in the gonad was more diverse in female spiny lobsters without eyestalk ablation com-
- 565 pared to female spiny lobsters with one and two eyestalk ablation. Harrison (1990) supported the results of this study that when maturation was induced by eyestalk ablation it would be able to accelerate hormonal and metabolic change that would stimulate ovarian
- 570 development to reach its peak. It means there is a low variety in nutrient but with high concentration for specific purposes such as egg yolk deposition.

Many new fatty acids class that were found in this study may improve our understanding and knowledge

575 to modify feed composition for brood stock reproduction in spiny lobster. In addition, these results will fill the gap of information required for further development of spiny lobster mariculture industry.

Disclosure statement

580 No potential conflict of interest was reported by the authors. AQ12

Funding

AQ4 This work was supported by the Doctoral Dissertation Research Grant from Ministry of Research, Technology and Higher Education of The Republic of Indonesia [No. 062/ SP2H/LT/DRPM/2018].

585 AQ5

590 AQ13

References

- Burns BG, Sangalang GB, Freeman HC, McMenemy M. 1984. Bioconversion of steroids by the testes of the American lobster, *Homarus americanus, in vitro*. Gen Comp Endocrinol. 54:422–428.
- Fairs NJ, Evershed RP, Quilan PT, Goad LJ. 1989. Detection of unconjucated and conjugated steroids in the ovary, eggs, and haemolymph of the decapod crustacean *Nephrops norvegicus*. Gen Comp Endocrinol. 4:199–208.
- Fairs NJ, Quinlan PT, Goad LJ. 1990. Changes in ovarian 595 unconjugated and conjugated steroid titers during vitellogenesis in *Penaeus monodon*. Aquaculture. 89:83–99.
- Fernandez F, Radhakhrisnan EV. 2016. Effect of bilateral eyestalk ablation on ovarian development and moulting in early and late intermoult stages of female spiny lobster *Panulirus homarus* (Linnaeus, 1758). Invertebrate Reprod Dev. 60:238–242.
- Garofalaki TF, Miniadis-Meimaroglou S, Sinanoglou VJ. 2006. Main phospholipids and their fatty acid composition in muscle and cephalothorax of the edible mediterranean crustacean 605 *Panulirus vulgaris* (spiny lobster). Chem Phys Lipids. 140:55–65.
- Ghalibaf A, Lehto J, Alén R. 2017. Fast pyrolysis of hot water-extracted and delignified silver birch (*Betula pendula*) sawdust by Py-GC/MS. J Anal Appl Pyrolysis. 127:17–22.
- Harrison KE. 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. J Shellfish Res. 9:1–28.
- Iromo H, Zairin M Jr, Suprayudi MA, Manalu W. 2014. Thyroxine distribution in the hemolymph, hepatopancreas, ovary, sponge, and larvae of female mud crabs (*Scylla serrata*) 615 during ovarian maturation. J Crustac Biol. 34:760–763.
- Kanazawa A, Teshima S. 1971. In vivo conversion of cholesterol to steroid hormones in the spiny lobster, *Panulirus japonica*. Bull Jpn Society of Sci Fish. 37:891–898.
- Kirubagaran R, Peter DM, Dharani G, Vinithkumar NV, 620 Sreeraj G, Ravindran M. 2005. Changes in vertebrate-type steroid and 5-hydroxytryptamine during ovarian recrudescence in the Indian spiny lobster, *Panulirus homarus*. N Z J Mar Freshwater Res 39:527–537.
- Kumar V, Sinha AK, Romano N, Allen KM, Bowman BA, 625 Thompson KR, Tidwell JH. 2018. Metabolism and nutritive role of cholesterol in the growth, gonadal development, and reproduction of crustaceans. Rev Fish Aquacult. 26:254–273.
- Martinez-Balmori D, Olivares F, Spaccini R, Aguiar KP, Araújo MF, Aguiar NO, Guridi F, Canellas LP. 2013. 630 Molecular characteristics of vermicompost and their relation to preservation of inoculated nitrogen-fixing bacteria. J Anal Appl Pyrolysis. 104:540–550.
- Nan FH, Wu YS, Chang NC. 2015. The effect of steroid hormone feeds on the reproductive biology of the spiny lobster, *Panulirus interruptus* (J.W. Randall, 1840) (Decapoda, Palinura). Crustaceana. 88:1367–1386.
- Nandi J. 1967. Comparative endocrinology of steroid hormones in vertebrates. Am Zool. 7:115–133.
- Pillai SM, Verghese PU, Ravichandran P, Roy AK. 1987. Effect of 640 thyroxine on growth and moulting in *Penaeus monodon* fabricius. Indian J Anim Sci. 57:241–245.

Quackenbush LS. 1994. Lobster reproduction: a review. Crustaceana. 67:82-94.

- 645 Radhakrishnan EV, Vijayakumaran M. 1984. Effect of eyestalk ablation in the spiny lobster Panulirus homarus (Linnaeus): 3. On gonadal maturity. Indian J Fish. 31:209-216.
 - Roustaian P, Gaik LA. 2006. Effect of thyroxine immersion on larval survival, growth and postlarvae production of fresh-
- 650 water prawn, Macrobrachium rosenbergii (de Man). Aquacult Res. 37:1378-1380.
 - Shields JD, Boyd R. 2014. Atlas of lobster anatomy and histology. Virginia: Virginia Institute of Marine Science.
 - Subramoniam T. 2011. Mechanism and control of vitellogenesis in crustaceans. Fish Sci. 77:1-21.
- 655
- Subramoniam T. 2017a. Sexual biology and reproduction in crustaceans. London: Academic Press.
- Subramoniam T. 2017b. Steroidal control of vitellogenesis in crustacean: a new understanding for improving shrimp hatchery
- production. Proceeding Indian National Sci Acad. 83:595–610. 660 Subramoniam T, Kirubagaran R. 2010. Endocrine regulation of vitellogenesis in lobsters. J Mar Biol Ass India. 52:229-236.

- Swevers L, Lambert JGD, De Loof A. 1991. Metabolism of vertebrate-type steroids by tissues of three crustacean species. Comp Biochem Physiol. 99B:35-41.
- Vijayakumaran M, Radhakrishnan EV. 1984. Effect of eyestalk ablation in the spiny lobster Panulirus homarus (Linnaeus): 2. On food intake and conversion. Indian J Fish. 31:148–155.
- Wilder MN, Okumura T, Tsutsui N. 2010. Reproductive 670 mechanism in crustacea focusing on selected prawn species: vitellogenin structure, processing and synthetic control. Agua-Biosci Monogr. 3:73-110.
- Yan H, Xue M, Liu H, Wang L, Liu O, Jiang L, 2017, Energy reserves and gonad steroid levels during the reproductive 675 cycle of Japanese mantis shrimp Oratosquilla oratoria De Haan, 1844 (Stomatopoda: squillidae) in pikou bay, dalian, China. J Crustac Biol. 37:99-108.
- Yano I. 1998. Hormonal control of vitellogenesis in penaeid shrimp. In: Flagel TW, editor. Advances in Shrimp biotech-680 nology. Thailand: National Center for Genetic Engineering and Biotechnology; p. 29-31.
- Young T, Alfaro AC. 2018. Metabolomic strategies for aquaculture research: a primer. Rev Aquacult. 10:26-56.

665