been completely sequenced to date, estimates are that the American lobster (*Homarus americanus*) genome is about 4.40 pg/4.3 Gbp or greater (see Animal Genome Size Database website and Gloucester Marine Genomics Institute website, American Lobster Genome, on the internet).

[007] The lack of information regarding shrimp, prawn, crab, crayfish, and/or lobster is arguably largely due to challenges in cell culture. For example, shrimp/prawn cell lines have only been established from the lymphoid organ and ovaries (Tapay *et al*. (1995) Proc Soc Exp Biol Med 209(1):73-8; Hsu *et al*. (1995) Aquaculture 136:43-55; US patent number 6143547; George and Dhar (2010) In Vitro Cell Dev Biol Anim 46(9):801-10; Ma *et al*. (2017) Reviews in Aquaculture 9: 88-98). In addition, shrimp/prawn primary muscle cultures have lasted only up to 12 days (George and Dhar, 2010) and no known primary cultures or cell lines have been described for fat cells.

[008] Furthermore, attempts to culture fat cells from shrimp, prawn, crab, crayfish, and/or lobster have not been described.

[009] The availability of immortalized cell lines from shrimp, prawn, crab, crayfish, and/or lobster would allow cellular agriculture companies to progress to the next steps of cellular agriculture (Rubio *et al*., (2019) Front. Sustain. Food Syst. doi: 10.3389/fsufs.2019.00043). Rather than the time consuming process of isolating primary cells, food regulatory and research groups would then have a ready source of cell lines. This would facilitate understanding shrimp, prawn, crab, crayfish, and/or lobster viral diseases, testing for the presence of those viruses, and developing treatments (Ma *et al*. (2017) Reviews in Aquaculture 9: 88-98). Consequently, there remains a need for immortalized muscle and fat cell lines from shrimp, prawn, crab, crayfish, and/or lobster. The disclosure presented herein addresses this need.

SUMMARY

[009a] In one aspect, the present disclosure refers to a ~~renewable~~ crustacean muscle stem cell line ~~comprising~~ obtained from isolated primary muscle stem cells, wherein the isolated primary muscle stem cells are selected from the group consisting of shrimp, prawn, lobster, crab, and crayfish.
[009b] In another aspect, the present disclosure refers to a crustacean tissue comprising ~~the~~

muscle cells differentiated from the ~~renewable~~ crustacean muscle stem cell line as described herein.

[009c] In another aspect, the present disclosure refers to a meat product comprising ~~the~~ muscle cells differentiated from the ~~renewable~~ crustacean muscle stem cell line as described herein.

[009d] In another aspect, the present disclosure refers to a method for producing a shrimp~~, prawn, crab, crayfish,~~ and/or ~~lobster~~ prawn ~~renewable~~ muscle stem cell line according to claim 1 comprising (a) isolating shrimp and/or~~,~~ prawn~~, crab, crayfish, and/or lobster~~ muscle ~~tissue~~; (b) sterilizing the isolated muscle ~~tissue~~ and ~~optionally~~ removing fat and epidermal tissue comprised in the muscle; (c) mincing the ~~sterilized~~ isolated muscle after step (b) ~~tissue~~to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise cells; (d) incubating the cell culture~~media and minced sections~~ of step (c) at 28°C without CO_2 for 3 to 7 days; (e) removing the ~~remaining~~ minced muscle tissue sections from the cell culture, wherein the cells comprised in the muscle tissue sections remain in the cell culture;~~,~~ (f) adding fresh media to the cell culture~~,~~ and incubating the ~~remaining~~ cells in the ~~media~~ cell culture at 28°C without CO_2 for 3 to 7 days; and (~~f~~g) repeating step (~~e~~f) when cells number reaches about 8 million in 10 ml of ~~culture~~ media, wherein step (f) is repeated at least 10 times.

[009e] In another aspect, the present disclosure refers to cell cultures comprising the ~~renewable~~ crustacean muscle stem cell lines as described herein.

[0009f] In another aspect, the present disclosure refers to a cell culture comprising the ~~renewable~~ crustacean muscle stem cell line as described herein, wherein the ~~renewable~~ crustacean muscle stem cell line is shrimp, and/or prawn muscle cells from a species belonging to the genus *Penaeus*, *Solenocera*, *Metapenaeus*, *Parapenaeus*, *Parapenaeopsis*, *Metapenaeopsis*, *Trachypenaeus*, *Protrachypene*, *Xiphopenaeus*, *Hymenopenaeus*, *Atypopenaeus*, *Eusicyonia*, *Sicyonia*, or *Litopenaeus*.

[009g] In yet another aspect, the present disclosure refers to a cell culture comprising the ~~renewable~~ crustacean muscle stem cell line as described herein, wherein the ~~renewable~~ crustacean muscle stem cell line is shrimp, and/or prawn muscle stem cells, wherein the shrimp, and/or prawn muscle stem cells are from *Penaeus monodon* and/or *Litopenaeus vannamei*.

[009h] In another aspect, the present disclosure refers to a method for producing a crab muscle stem cell line according to claim 1 comprising (a) isolating crab muscle from claws, legs, and

bodies of freshly sacrificed crabs; (b) sterilizing the isolated muscle and removing fat and epidermal tissue comprised in the muscle; (c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells; (d) incubating the cell culture of step (c) at 20-24^{o}C without CO_2 for about 7 days; (e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture; (f) adding fresh media to the cell culture and incubating the cells in the cell culture at 20-24^{o}C without CO_2; and (g) repeating step (f) when cells number reaches about 8 million in 10 ml of media, wherein step (f) is repeated at least 10 times.

[009i] In another aspect, the present disclosure refers to a method for producing a crayfish or lobster muscle stem cell line according to claim 1 comprising (a) isolating crayfish or lobster muscle from claws, legs, and tails of freshly sacrificed crayfish or lobster; (b) sterilizing the isolated muscle and removing fat and epidermal tissue comprised in the muscle; (c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells; (d) incubating the cell culture of step (c) at 27^{o}C without CO_2 for about 7 days for crayfish or at saturated humidity at 5^{o}C for lobster; (e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture; (f) adding fresh media to the cell culture and incubating the cells in the cell culture at 27^{o}C without CO_2 for crayfish or at saturated humidity at 5^{o}C for lobster; and (g) repeating step (f) when cells number reaches about 8 million in 10 ml of media, wherein step (f) is repeated at least 10 times.

[010] The present disclosure is directed to tissue engineered shrimp, prawn, crab, crayfish, and/or lobster meat products and methods for producing such products. In one example, the meat product comprises muscle cells that are grown *ex vivo* and/or *in vitro*. In a further example, the

CLAIMS

We claim:

1. A ~~renewable~~ crustacean muscle stem cell line ~~comprising~~ obtained from isolated primary muscle stem cells, wherein the isolated primary muscle stem cells are selected from the group consisting of shrimp, prawn, lobster, crab, and crayfish.

2. The ~~renewable~~ crustacean muscle stem cell line according to claim 1, wherein the cell line has completed at least 10 passages.

~~3. The renewable crustacean cell line according to claim 1, wherein the primary muscle cells are stem cells.~~

34. The ~~renewable~~ crustacean muscle stem cell line according to claim 1, wherein the ~~primary~~ muscle stem cells differentiate into myofibers.

~~5. The renewable crustacean muscle cell line of claim 1, wherein the primary muscle cells are immortalized primary muscle cells.~~

~~6. The renewable crustacean muscle cell line of claim 1, wherein the primary muscle cells are selected from the group consisting of shrimp, prawn, lobster, crab, and crayfish.~~

47. The ~~renewable~~ crustacean muscle stem cell line of claim 1, wherein the isolated primary muscle stem cells are shrimp or lobster primary muscle stem cells.

85. A crustacean tissue comprising ~~the~~ muscle cells differentiated from the ~~renewable~~ crustacean muscle stem cell line of claim 1.

96. A meat product comprising ~~the~~ muscle cells differentiated from the ~~renewable~~ crustacean muscle stem cell line of claim 1.

7~~10~~. The meat product according to claim ~~9~~6~~-~~, further comprising other shrimp, prawn, crab, crayfish, and/or lobster cells.

~~11~~8. The meat product according to claim ~~9~~6, wherein the ~~renewable~~ crustacean muscle stem cell line is shrimp~~,~~ and/or prawn muscle cells from a species belonging to the genus *Penaeus*, *Solenocera*, *Metapenaeus*, *Parapenaeus*, *Parapenaeopsis*, *Metapenaeopsis*, *Trachypenaeus*, *Protrachypene*, *Xiphopenaeus*, *Hymenopenaeus*, *Atypopenaeus*, *Eusicyonia*, *Sicyonia*, or *Litopenaeus.*

~~12~~9. The meat product according to claim 6~~9~~, wherein the ~~renewable~~ crustacean muscle stem cell line is shrimp~~,~~ and/or prawn muscle cells from *Penaeus monodon* and/or *Litopenaeus vannamei*.

~~13~~10. A method for producing a shrimp~~, prawn, crab, crayfish,~~ and/or ~~lobster~~ prawn ~~renewable~~ muscle stem cell line according to claim 1 comprising

(a) isolating shrimp and/or~~,~~ prawn~~, crab, crayfish, and/or lobster~~ muscle ~~tissue~~;

(b) sterilizing the isolated muscle ~~tissue~~ and ~~optionally~~ removing fat and epidermal tissue comprised in the muscle;

(c) mincing the ~~sterilized~~ isolated muscle after step (b) ~~tissue~~to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise cells;

(d) incubating the cell culture~~media and minced sections~~ of step (c) at 28ºC without CO_2 for 3 to 7 days;

(e) removing the ~~remaining~~ minced muscle tissue sections from the cell culture, wherein the cells comprised in the muscle tissue sections remain in the cell culture;~~,~~

(f) adding fresh media to the cell culture~~,~~ and incubating the ~~remaining~~ cells in the ~~media~~ cell culture at 28ºC without CO_2 for 3 to 7 days; and

(~~f~~g) repeating step (~~e~~f) when cells number reaches about 8 million in 10 ml of ~~culture~~ media,

wherein step (f) is repeated at least 10 times.

~~14~~11. A cell culture comprising the ~~renewable~~ crustacean muscle stem cell line according to claim 1.

~~15~~12. A cell culture comprising the ~~renewable~~ crustacean muscle stem cell line of claim 3.

~~16~~13. A cell culture comprising the ~~renewable~~ crustacean muscle stem cell line according to claim 4.

~~17. A cell culture comprising the renewable crustacean muscle cell line according to claim 7.~~

14. A method for producing a crab muscle stem cell line according to claim 1 comprising

(a) isolating crab muscle from claws, legs, and bodies of freshly sacrificed crabs;

(b) sterilizing the isolated muscle and removing fat and epidermal tissue comprised in the muscle;

(c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections, and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells;

(d) incubating the cell culture of step (c) at 20-24°C without CO_2 for about 7 days;

(e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture;

(f) adding fresh media to the cell culture and incubating the cells in the cell culture at 20-24°C without CO_2; and

(g) repeating step (f) when cells number reaches about 8 million in 10 ml of media, wherein step (f) is repeated at least 10 times.

15. A method for producing a crayfish or lobster muscle stem cell line according to claim 1 comprising

(a) isolating crayfish or lobster muscle from claws, legs, and tails of freshly sacrificed crayfish or lobster;

(b) sterilizing the isolated muscle and removing fat and epidermal tissue comprised in the muscle;

(c) mincing the isolated muscle after step (b) to obtain minced muscle tissue sections,

and placing the minced muscle tissue sections into a cell culture with a cell culture media, wherein the minced muscle tissue sections comprise muscle stem cells;

(d) incubating the cell culture of step (c) at 27°C without CO_2 for about 7 days for crayfish or at saturated humidity at 5°C for lobster;

(e) removing the minced muscle tissue sections from the cell culture, wherein the muscle stem cells comprised in the muscle tissue sections remain in the cell culture;

(f) adding fresh media to the cell culture and incubating the cells in the cell culture at 27°C without CO_2 for crayfish or at saturated humidity at 5°C for lobster; and

(g) repeating step (f) when cells number reaches about 8 million in 10 ml of media, wherein step (f) is repeated at least 10 times.

~~18~~16. A cell culture comprising the ~~renewable~~ crustacean muscle stem cell line according to claim 1, wherein the ~~renewable~~ crustacean muscle stem cell line is shrimp, and/or prawn muscle cells from a species belonging to the genus *Penaeus*, *Solenocera*, *Metapenaeus*, *Parapenaeus*, *Parapenaeopsis*, *Metapenaeopsis*, *Trachypenaeus*, *Protrachypene*, *Xiphopenaeus*, *Hymenopenaeus*, *Atypopenaeus*, *Eusicyonia*, *Sicyonia*, or *Litopenaeus*.

~~19~~17. A cell culture comprising the ~~renewable~~ crustacean muscle stem cell line according to claim 1, wherein the ~~renewable~~ crustacean muscle stem cell line is shrimp, and/or prawn muscle stem cells, wherein the shrimp, and/or prawn muscle stem cells are from *Penaeus monodon* and/or *Litopenaeus vannamei*.