Marine Genomics 33 (2017) 17–19

Contents lists available at ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen

De novo transcriptome assembly of the amphipod *Gammarus chevreuxi* exposed to chronic hypoxia

Michael Collins, Oliver Tills, John I Spicer, Manuela Truebano *

Marine Biology and Ecology Research Centre, School of Biological and Marine Sciences, Plymouth University, Drake Circus, Plymouth PL4 8AA, UK

ARTICLE INFO

Article history:
Received 12 January 2017
Received in revised form 20 January 2017
Accepted 26 January 2017
Available online 8 February 2017

Keywords:
Transcriptome
Chronic hypoxia
Gammarus
Amphipod

ABSTRACT

Environmental hypoxia is becoming more prevalent in aquatic environments due to eutrophication and climate change. While the ecological and physiological responses of marine animals to hypoxia have received considerable attention, the molecular responses remain largely undetermined. We have assembled a transcriptome for the brackishwater amphipod, *Gammarus chevreuxi*, exposed to three different levels of environmental oxygen (100, 40 and 20% air saturation). Sequencing using Illumina HiSeq 2000 produced 227.1 M reads which were assembled into 291,934 contigs corresponding to 218,558 genes. The assembled transcriptome provides a valuable resource to explore the molecular mechanisms underpinning responses to chronic hypoxia in an ecologically-important aquatic invertebrate.

1. Introduction

Animals inhabiting shallow coastal regions are increasingly being subjected to prolonged episodes of low oxygen (chronic hypoxia) driven by the combined effects of climate change and eutrophication (Diaz and Rosenberg, 2008; Rabalais et al., 2014; Altieri and Gedan, 2015). Chronic hypoxia can result in ecological restructuring of communities and mass mortality of sensitive organisms (Diaz and Rosenberg, 1995), but some hypoxia-tolerant species are able to make adjustments at both the physiological and molecular level to promote survival (Hochachka et al., 1996; Spicer, 2016). While the physiological responses of marine animals to low oxygen have been well documented (Spicer, 2016), the underlying molecular mechanisms have received little attention, particularly for marine invertebrates (Spicer, 2014). Gammarid amphipods are abundant in coastal and estuarine areas where they play important functional roles (Lincoln, 1979). They are excellent models for investigating molecular responses to hypoxia, as physiological responses are relatively well understood (Bulnheim, 1979; Agnew and Taylor, 1985; Agnew and Jones, 1986; Hoback and Barnhart, 1996; Hervant et al., 1999; Spicer et al., 2002). As emerging model organisms for ecotoxicology (*e.g.* Chaumot et al., 2015) and developmental biology (Wolff and Gerberding, 2015), amphipods have received renewed interest. Accordingly, the availability of genomic resources for amphipods is increasing, with transcriptomic data now available for a few amphipod species (Zeng et al., 2011; Gismondi and Thomé, 2016; Truebano et al., 2016; Ford et al., 2008). The aim of this study is to assemble a transcriptome for hypoxic *G. chevreuxi* that can be further explored to investigate the molecular mechanisms underpinning the responses to chronic hypoxia, as well as adding to the growing body of genomic resources for this species.

2. Data description

2.1. Hypoxia exposure and library preparation

Gammarus chevreuxi were collected from the River Plym, Plymouth (−50° 39′ 03″ N, 4° 08′ 56″ W) and acclimated to laboratory conditions (T = 15 °C, S = 15, 12 h L:12 h D regime) for a minimum of 4 weeks prior to experimentation. They were fed carrot *ad libitium*. Only adult males were used in this study to remove the effects of life cycle and gender. Individuals were exposed to normoxia (100% air saturation), moderate hypoxia (40% air saturation), or severe hypoxia (20% air saturation) for 1 week. This was achieved using a mesocosm system consisting of 16 sealed aquaria (vol. = 0.48 L, T = 14.52 °C, S = 15, eight aquaria per treatment, 15 animals in each). Normoxic aquaria were aerated using an air pump (Mistral 2000, Aqua Medic GmbH, Germany). Aquaria water was made hypoxic by bubbling with a gas mixture of nitrogen and air controlled using adjustable flow valves (Platon NG series glass flowmeter 0–10 L min^{-1}, CT Platon, France; Flowmeter RA609325, KDG, UK). Due to the design of the mesocosm system, the effects of moderate and severe hypoxia were investigated in separate experiments. Upon removal individuals were frozen in liquid nitrogen and stored at −80 °C. Total RNA was extracted from three pools of animals per treatment (n = 10) using the PureLink RNA Mini Kit (Ambion, USA) with a TRIzol step. RNA integrity was

* Corresponding author.
E-mail addresses: michael.collins@plymouth.ac.uk (M. Collins), oliver.tills@plymouth.ac.uk (O. Tills), J.I.Spicer@plymouth.ac.uk (J.I. Spicer), manuela.truebanogarcia@plymouth.ac.uk (M. Truebano).

http://dx.doi.org/10.1016/j.margen.2017.01.006
1874-7787/

Table 1
MixS descriptors.

Item	Description
Investigation_type	Eukaryote
Project_name	Adult transcriptome for *Gammarus chevreuxi*
Lat_lon	−50° 39′ 03″ N, 4° 08′ 56″ W
Geo_loc_name	United Kingdom: Plymouth
Collected_by	Manuela Truebano
Collection_date	01-Jun-13
Environment	Brackish estuary
Biome	ENVO:00002137
Feature	ENVO:00000229
Material	ENVO:00002019
Depth	<0.5 m
Alt-elev	0 m
Temperature	15 °C
Salinity	15 PSU
Sequencing method	Illumina HiSeq
Assembly method	Trinity (v 2.2.0)
Assembly name	*Gammarus chevreuxi* adult transcriptome
Genome coverage	×10

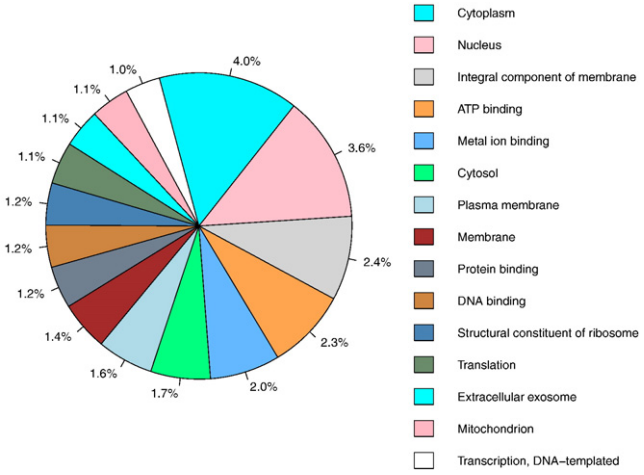

Fig. 1. Transcripts mapping to the top 15 GO terms expressed as a percentage of all transcripts generated by the assembly.

determined using a Bioanalyzer (Agilent Technologies, USA). TruSeq RNA libraries (Illumina, San Diego, USA) were synthesised and sequenced on a single lane of an Illumina HiSeq 2000 using 100 base paired-end sequencing (HiSeq 2000, Illumina, San Diego, USA). MixS descriptors are presented in Table 1.

2.2. Assembly and annotation

Sequencing produced 227.1 M 100 bp paired-end reads. *De novo* transcriptome assembly was performed using the Trinity pipeline (v 2.2.0, with the parameters –trimmomatic, for adapter trimming, and –normalise reads, for digital normalisation) (Haas et al., 2013). Contigs were annotated using Trinotate (v 3.0.0, www.trinotate.github.io) with an e-value cut-off of 1e-05 (Table 2). Transcriptome assembly contained 291,934 contigs assigned to 218,558 genes (Trinity genes). Filtering of the assembly (FPKM > 1 and Isopct > 1) reduced complexity to 144,501 sequences corresponding to 107,528 genes. Within the assembly, 24,030 transcripts contained gene ontology (GO) term annotations (Supplementary File S1). The assembled transcripts corresponded to 12,400 unique GO terms (Supplementary File S2). The top 15 GO terms and the percentage of transcripts mapped to each term are presented (Fig. 1).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2017.01.006.

Data deposition

Assembled contigs (TSA project accession number GFCV01000000, sequences GFCV01000001-GFCV01144501) and raw reads (SRA: SRR5109797- SRR5109805) have been deposited in the European Nucleotide Archive, Bioproject Number "PRJNA357029".

Table 2
Assembly statistics.

Assembled bases	Number of contigs	Mean contig length	Median contig length	N50	GC content
117,494,825	144,501	813.11	350	1618	43.94

Annotation statistics (transcripts)

Swissprot (blastx)	SignalP	GO	Eggnog	KEGG
23,498	2902	24,030	17,852	18,833

Acknowledgements

Sequencing was performed at The Genome Analysis Centre, Norwich. This work was funded by a grant from the School of Marine Sciences and Engineering, Plymouth University.

References

Agnew, D.J., Jones, 1986. Metabolic adaptations of *Gammarus duebeni* liljeborg (Crustacea, Amphipoda) to hypoxia in a sewage treatment plant. Comp. Biochem. Physiol. A Physiol. 84 (3), 475–478.
Agnew, D.J., Taylor, A.C., 1985. The effect of oxygen tension on the physiology and distribution of *Echinogammarus pirloti* (Sexton & Spooner) and *E. obtusatus* (Dahl) (Crustacea: Amphipoda). J. Exp. Mar. Biol. Ecol. 87 (2), 169–190.
Altieri, A.H., Gedan, K.B., 2015. Climate change and dead zones. Glob. Chang. Biol. 21 (4), 1395–1406.
Bulnheim, H.P., 1979. Comparative studies on the physiological ecology of five euryhaline *Gammarus* species. Oecologia 44 (1), 80–86.
Chaumot, A., Geffard, O., Armengaud, J., Maltby, L., 2015. Gammarids as reference species for freshwater monitoring. In: Amiard-Triquet, C., Amiard, J.C., Mouneyrac, C. (Eds.), Aquatic Ecotoxicology. Advancing Tools for Dealing With Emerging Risks. Academic Press, London, pp. 253–280 (Chapter 11).
Diaz, R.J., Rosenberg, R., 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanogr. Mar. Biol. Annu. Rev. 33, 245–303.
Diaz, R.J., Rosenberg, R., 2008. Spreading dead zones and consequences for marine ecosystems. Science 321, 926–929.
Ford, A., Shambles, C., Kille, P., 2008. Intersexuality in crustaceans: genetic, individual and population effects. Mar. Environ. Res. 66 (1), 146–148.
Gismondi, E., Thomé, J.P., 2016. Transcriptome of the freshwater amphipod *Gammarus pulex* hepatopancreas. Genomics Data. 8, pp. 91–92.
Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., et al., 2013. *De novo* transcript sequence reconstruction from RNA-Seq: reference generation and analysis with Trinity. Nat. Protoc. 8 (8), 1494–1512.
Hervant, F., Mathieu, J., Culver, D.C., 1999. Comparative responses to severe hypoxia and subsequent recovery in closely related amphipod populations (*Gammarus minus*) from cave and surface habitats. Hydrobiologia 392 (2), 197–204.
Hoback, W., Barnhart, M., 1996. Lethal limits and sublethal effects of hypoxia on the amphipod *Gammarus pseudolimnaeus*. J. N. Am. Benthol. Soc. 15 (1), 117–126.
Hochachka, P.W., Buck, L.T., Doll, C.J., Land, S.C., 1996. Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. Proc. Natl. Acad. Sci. 93 (18), 9493–9498.
Lincoln, R.J., 1979. British Marine Amphipoda: Gammaridea. British Museum (Natural History), London.
Rabalais, N.N., Cai, W.J., Carstensen, J., Conley, D., et al., 2014. Eutrophication-driven deoxygenation in the coastal ocean. Oceanography 27 (1), 172–183.
Spicer, J.I., 2014. What can an ecophysiological approach tell us about the physiological responses of marine invertebrates to hypoxia? J. Exp. Biol. 217, 46–56.
Spicer, J.I., 2016. Respiratory responses of marine animals to environmental hypoxia. In: Solan, M., Whiteley, N.M. (Eds.), Stressors in the Marine Environment. Physiological

and Ecological Responses; Societal Implications. Oxford University Press, Oxford, pp. 25–35 (Chapter 2).
Spicer, J.I., Dando, C., Maltby, L., 2002. Anaerobic capacity of a crustacean sensitive to low environmental oxygen tensions, the freshwater amphipod *Gammarus pulex* (L.). Hydrobiologia 477, 189–194.
Truebano, M., Tills, O., Spicer, J.I., 2016. Embryonic transcriptome of the brackishwater amphipod *Gammarus chevreuxi*. Mar. Genomics 28, 5–6.
Wolff, C., Gerberding, M., 2015. Crustacea: comparative aspects of early development. In: Wanninger, A. (Ed.), Evolutionary Developmental Biology of Invertebrates. 4 Ecdysozoa II. Crustacea, 2. Springer-Verlag, Wien, pp. 39–61.
Zeng, V., Villanueva, K.E., Ewen-Campen, B.S., Alwes, F., Browne, W.E., Extavour, C.G., 2011. De novo assembly and characterization of a maternal and developmental transcriptome for the emerging model crustacean *Parhyale hawaiensis*. BMC Genomics 12, 581.