Two *de novo* transcriptome assemblies and functional annotations from juvenile cuttlefish (*Sepia officinalis*) under various metal and *p*CO2 exposure conditions

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ABSTRACT

The cuttlefish *Sepia officinalis* is a precious model in behavioural and neurobiology studies. It is currently facing combined environmental changes related to the anthropogenic global change. However, genomic resources available to support investigations tackling this issue are still scarce. Therefore, we present two annotated *de novo* transcriptome assemblies from recently hatched (whole body) and one-month-old (head) *Sepia officinalis* juveniles. Both assemblies rely on an important read depth validated by a pseudo-rarefaction analysis, and gathered several individuals from various metal and pCO_2 exposure conditions. After redundancy reduction, assemblies from newly hatched and one-month-old individuals comprised 244,772 and 397,569 transcripts with 39,804 and 49,402 putative ORFs, respectively, and an average annotation rate of 60%. Assemblies were compared to each other, revealing age-specific transcriptomic landscapes. These two assemblies constitute highly valuable genomic resources facilitating the investigation of transcriptomic endpoints in environmental studies considering coleoid cephalopods.

Keywords: Bioinformatics, Transcriptome announcement, Cephalopods, Ecotoxicology, Non-model species

Introduction

Cephalopods constitute a remarkable class within molluscs, exhibiting an outstanding diversity of morphologies, habitats and ecology. They also display some traits thought to be the result of convergent evolution with vertebrates (*i.e.* camera-like eye and closed circulatory system). Cephalopods are divided in two subclasses: Nautiloidea Blainville, 1825 and Coleoidea Bather, 1888. While the former contains only a few living fossil species, the latter, counting more than 800 species, represents the large majority of extant cephalopods (*c.a.* octopuses, cuttlefishes, squids), characterized by a complex central nervous system somewhat analogous to those of vertebrates (Packard, 1972).

So far mostly considered in behaviour and neurobiology studies, cephalopods are becoming promising model systems in ecotoxicology, for biomonitoring (Ajala et al., 2022) as well as experimental studies (García-Flores et al., 2025; Gouveneaux et al., 2023; Penicaud et al., 2017). All these research areas gained efficiency from the recent development of "-omics" technologies, promoting cephalopod genomic studies (Albertin & Simakov, 2019; Baden et al., 2023). Indeed, several transcriptomes from diverse tissue types (*i.e. Octopus vulgaris* whole body, early life stages (Prado-Álvarez et al., 2022); *Idiosepius pygmaeus* central nervous system and eyes (Thomas et al., 2024)) along with genome assemblies (*i.e. O. vulgaris* (Destanović et al., 2023), *Amphioctopus fangsiao* (Jiang et al., 2022)) were released in the last years.

Among coleoid cephalopods, the cuttlefish *Sepia officinalis* is of great economic interest, particularly in the northern east Atlantic where it is one of the most abundant and harvested species (Arkhipkin et al., 2021). Besides, *S. officinalis* from different areas were shown to be chronically contaminated by various metals such as silver, lead, cadmium or mercury (Ahmed et al., 2022; Ajala et al., 2022; Ihya et al., 2021; Minet et al., 2021; Miramand et al., 2006), as well as anti-depressant compounds like venlafaxine or fluoxetine (Chabenat, Bidel, et al., 2021; Chabenat, Knigge, et al., 2021) leading to behavioural alterations and neurotoxic effects (see Lacoue-Labarthe et al. (2016) for a focus on early-life stages) potentially amplified by ocean acidification (see Minet (2022) for the example of combined exposure with mercury). Despite its strong scientific and commercial interest, there is still a lack of genomic resources for this species. Only one transcriptome assembly was released (posterior salivary gland (Gonçalves et al., 2024)) and the first chromosome-level reference genome, about to be published (Rencken et al., 2025), was annotated using species from mollusks families other than Sepiidae, to which *Sepia* belongs.

In this context, we present two *de novo* transcriptome assemblies built from the whole body of newly hatched juveniles (project "rnasep1") and head tissues of one-month-old juveniles (project "rnasep2") *S. officinalis* reared in various metal and pCO_2 conditions. The gathering of individuals from different experimental conditions enabled the provision of valuable ready-made genomic resources, facilitating future gene expression analyses and comparative transcriptomic studies in coleoid cephalopods.

Methods

Description of the biological material

Freshly laid eggs of common cuttlefish (*Sepia officinalis*) were collected in the Minimes Port, Bay of Biscay, France (46°08'44.7"N; 1°10'16.4"W) during the Spring of 2016 (rnasep1) and 2021 (rnasep2).

Newly hatched juveniles

Upon field collection, eggs were immediately transported to the laboratory. They were individualized from their clutches and then placed in eighteen 9 L glass aquaria (n=30 eggs per aquarium; salinity: 34.4 ppt; temperature: 18.4 ± 0.6°C; 12 h light:12 h dark cycle, with aeration, recirculation pumps). After 5 days of acclimation, nine aquaria were maintained at ambient seawater pH (pHT = 7.95 ± 0.03) and the nine others were gradually acidified by bubbling CO_2

until reaching a pHT of 7.70 ± 0.08. The pCO_2 regulation was handled by an IKS system from Aquastar© and weekly calibration of IKS probes with NBS standard pH solutions (pH 4.0 and 7.0), a glass electrode and using TRIS buffer solutions (salinity 35, provided by A. Dickson, Scripps Institution of Oceanography, San Diego, USA). Among each pH condition, two set of three aquaria were spiked with $AgNO_3$ or $HgCl_2$ solutions in order to reach 1 $\mu g.L^{-1}$ of metal in seawater, respectively. The last three aquaria were kept metal-free for control conditions. This resulted in 6 rnasep1 experimental conditions: Control (hereon "CT"), acidification ("pCO_2"), exposed to $HgCl_2$ ("$HgCl_2$"), exposed to $AgNO_3$ ("$AgNO_3$"), exposed to both acidification and $HgCl_2$ ("pCO_2 + $HgCl_2$") or exposed to acidification and $AgNO_3$ ("pCO_2+ $AgNO_3$") (Figure 1a). Water renewal was done every two days and spikes of metals were renewed accordingly to maintain the metal concentrations as constant as possible.

Eggs were maintained in these conditions for 40 days, until hatching. When hatched (considering a daily survey), juveniles were immediately collected, anaesthetized with 17.5 $mg.L^{-1}$ and eventually put to death using 2% ethanol in seawater. Then, individuals (n = 6 per condition) were frozen in liquid nitrogen and stored at -80°C before RNA extraction. Additionally, fourteen hatchlings were sampled for measurement of Ag and Hg concentrations in whole body to validate the exposure conditions (data available in sample description at ENA PRJEB89715).

One-month-old juveniles

Eggs were acclimated in the laboratory and raised until one-month old juvenile stage to investigate the effects of pCO_2 and MeHg on behaviour of cuttlefish early life stages (Minet, 2022). Briefly, eggs were individualized from their clutches and then acclimated for two weeks in four black plastic basins (*i.e.* batches) in control conditions (n=70 eggs per batch; salinity: 35.0 ppt; temperature: 18.3 ± 0.2°C; pH_{NBS}: 8.0 ± 0.1; 12 h light:12 h dark cycle, with aeration, recirculation pumps and daily water renewal). After the acclimation period, two batches remained in control conditions (pH_{NBS}: 8.03 ± 0.05 in 2020 and 8.01 ± 0.05 in 2021, equivalent to ~440 µatm), while the two others were gradually acclimated to acidified conditions by reducing pH_{NBS} (CO_2 bubbling) from 8.01 to 7.54 ± 0.06 (equivalent to ~1600 µatm). The pCO_2 regulation was handled by an IKS system from Aquastar© (pH measurements every 20 min and weekly calibration of IKS probes with NBS standard pH 4.0 and 7.0 solutions and a glass electrode.

Immediately after hatching, 27 individuals from each pH condition were transferred into six 20 L black plastic basins and individualized in cylindrical baskets of 60 mm width (*c.a.* 9 indiv. per batch in triplicate). Thereafter, juvenile cuttlefishes were fed *ad libitum* with control (half of the individuals) or MeHg-contaminated (other half of the individuals) live ditch shrimp (*Palaemon varians*). For the MeHg-contaminated condition, only the first shrimp of each day was contaminated to ensure the number of contaminated shrimps eaten to be the same for all MeHg-exposed individuals. This experiment resulted in 4 distinct conditions: Control (hereon "CT"), Acidification ("pCO_2"), contaminated by methyl-mercury ("MeHg") or a combination of acidification and methyl-mercury contamination ("pCO_2+MeHg") (Figure 1b).

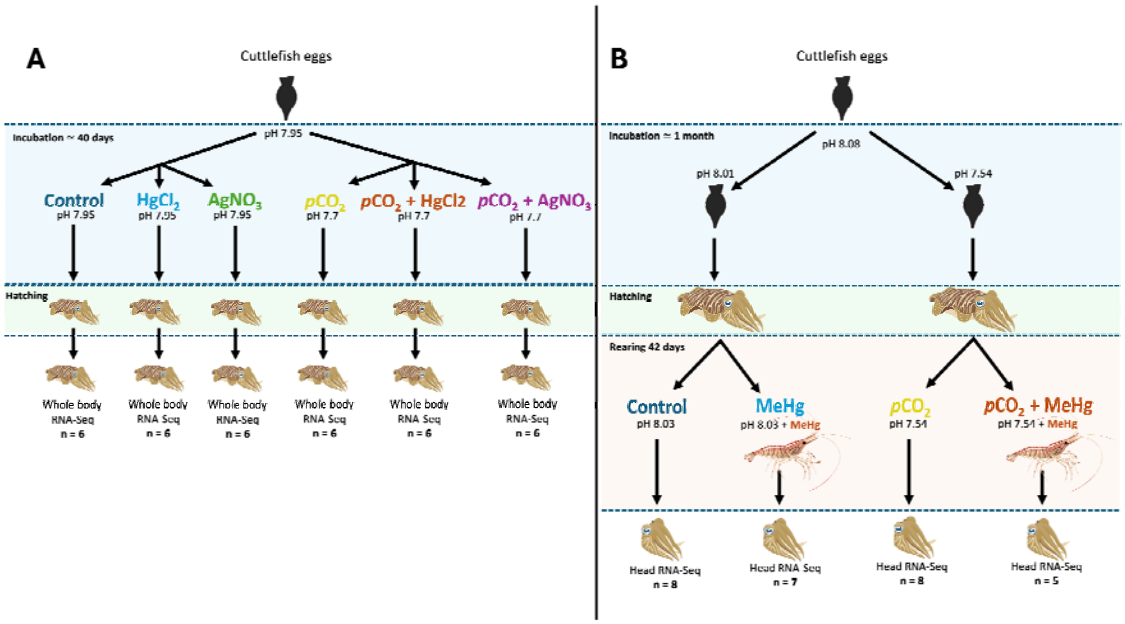

Figure 1 - Experimental design for A) newly hatched ("rnasep1"; carried out in 2016) and B) one-month-old juveniles ("rnasep2"; carried out in 2021) exposure to heavy metals and hypercapnia.

Hatchlings were raised for 42 days before being anaesthetized with 17.5 mg.L^{-1} MgCl^{2} and eventually sacrificed using 2% ethanol in seawater. In order to investigate the transcriptomic responses in the whole nervous central system to the experimental conditions, the heads of half of the individuals (CT: n=8; pCO_2: n=8; MeHg: n=7; pCO_2+MeHg: n=5) were immediately sampled and freeze dried in liquid nitrogen before storage at -80°C until RNA extraction. Total mercury concentrations were measured in the muscle of mantle to validate the dietary exposure (data available in sample description at ENA PRJEB88225).

The experiments were performed in accordance with the French and the European regulations (Directive 2010/63/EU) for the use of animals for scientific purposes. The experimental procedures were referenced and authorized by the French Ministry of Higher Education and Research for the use of animals for scientific purposes (APAFIS # 20520-2019050614554709).

RNA extraction and Illumina sequencing

Newly hatched juveniles

RNA extraction was performed using the Nucleospin for NucleoZOL (Macherey Nagel©), using manufacturer instructions. Whole individuals were first ground in liquid nitrogen. The resulting power was incubated in NucleoZOL + RNase-free water (>700 µL/50 mg of tissue powder) for 15 min after 15 s of vigorous vortexing. A 700 µL aliquot was then used for downstream RNA extraction steps. RNA was eluted in 60 µL RNase-free H_2O. A 5 µL aliquot was sampled for quantification on Qubit (using Thermo Fisher Scientific© HS and BR quantification kits) and 1% gel electrophoresis; the rest of the RNA extraction was immediately placed at -80°C until next steps. Further quality control on a Fragment Analyser (Agilent©), library construction and Illumina sequencing were outsourced to GeT-PlaGe platform from GenoToul, using the Illumina TruSeq Stranded mRNA Library Prep kit and an Illumina HiSeq 3000 platform (2x150bp).

Table 1 - Sequencing statistics for newly hatched juveniles. GeT-PlaGe sample code is used for the labelling of reads in the European Nucleotide Archive

repository. R1 GC% and R2 GC% are percent GC content on the forward and reverse reads, respectively.

GeT-PlaGe Sample Code	Sample Name	Number of Paired-end Reads	R1 GC%	R2 GC%
Ag771	SEP1-Ag7.7_1	21,283,196	40	41
Ag8-1-102	SEP1-Ag8.1_102	21,080,270	41	41
Ag7-7-29	SEP1-Ag7.7_29	17,316,330	41	41
Ag772	SEP1-Ag7.7_2	19,177,260	41	41
Ag7-7-31	SEP1-Ag7.7_31	22,963,224	41	41
Ag7-7-34	SEP1-Ag7.7_34	20,399,022	41	41
Ag773	SEP1-Ag7.7_3	17,805,326	41	41
Ag8-1-21	SEP1-Ag8.1_21	23,068,454	41	41
Ag8-1-27	SEP1-Ag8.1_27	21,202,072	40	41
Ag812	SEP1-Ag8.1_2	19,480,720	41	41
Ag813	SEP1-Ag8.1_3	20,526,026	41	41
Ag816	SEP1-Ag8.1_6	21,223,080	41	41
Hg7-7-125	SEP1-Hg7.7_125	24,256,850	41	41
Hg771	SEP1-Hg7.7_1	22,357,978	40	41
Hg772	SEP1-Hg7.7_2	19,811,618	40	41
Hg7-7-48	SEP1-Hg7.7_48	20,937,382	41	41
Hg7-7-49	SEP1-Hg7.7_49	19,721,682	41	41
Hg7-7-52	SEP1-Hg7.7_52	19,410,228	41	41
Hg8-1-111	SEP1-Hg8.1_111	21,700,826	41	42
Hg811	SEP1-Hg8.1_1	17,635,966	40	41
Hg812	SEP1-Hg8.1_2	24,195,958	40	40
Hg8-1-35	SEP1-Hg8.1_35	20,576,074	41	41
Hg8-1-36	SEP1-Hg8.1_36	19,962,954	40	40
Hg816	SEP1-Hg8.1_6	20,235,544	41	41
CT7-7-15	SEP1-CT7.7_15	18,380,114	40	41
CT7-7-18	SEP1-CT7.7_18	18,430,516	41	42
CT772	SEP1-CT7.7_2	17,710,208	40	40
CT773	SEP1-CT7.7_3	16,961,648	40	40
CT774	SEP1-CT7.7_4	15,762,346	40	40
CT7-7-93	SEP1-CT7.7_93	18,755,500	41	41
CT811	SEP1-CT8.1_1	20,027,126	40	41
CT812	SEP1-CT8.1_2	18,261,584	41	41
CT813	SEP1-CT8.1_3	18,859,762	41	41
CT8-1-7	SEP1-CT8.1_7	19,050,674	41	41
CT8-1-82	SEP1-CT8.1_82	20,522,436	41	41
CT8-1-9	SEP1-CT8.1_9	17,506,642	40	41

One-month-old juveniles

The RNA extraction and library preparation were outsourced to Fasteris©. Briefly, 28 heads underwent manual grinding in liquid nitrogen and shredded tissues were then preserved in RLT and beta-mercaptoethanol. Total RNA was isolated using the RNeasy 96 kit (Qiagen©, ref 74181) with final elution in 50 µL of nuclease-free water. After the extraction step, samples were quantified by Quant-iT (Thermo Fisher Scientific©) and the quality of RNA was checked by loading 2 µL of a dilution on a Fragment Analyzer (Agilent©). All samples reached the required concentration of 250-500 ng in a total volume of 50 µL. After poly(A) tail selection, libraries were constructed using the Illumina TruSeq Stranded mRNA Library Prep kit with NEXTFLEX unique dual index barcodes (Revvity©). The libraries were sequenced on an Illumina NovaSeq 6000 platform (2x150bp).

Table 2 - Sequencing statistics for one-month-old juveniles. Fasteris sample code is used for the labelling of reads in the European Nucleotide Archive repository. R1 GC% and R2 GC% are percent GC content on the forward and reverse reads, respectively.

Fasteris Sample Code	Sample Name	Number of Paired-end Reads	R1 GC%	R2 GC%
AVRX-1	SEP2-CT8.1_5	31,819,911	42	42
AVRX-2	SEP2-CT8.1_6	37,005,211	43	43

Fasteris Sample Code	Sample Name	Number of Paired-end Reads	R1 GC%	R2 GC%
AVRX-3	SEP2-CT8.1_8	29,634,371	42	43
AVRX-4	SEP2-CT8.1_9	33,148,390	42	43
AVRX-5	SEP2-CT8.1_37	25,239,430	42	42
AVRX-6	SEP2-CT8.1_38	31,105,749	43	43
AVRX-7	SEP2-CT8.1_39	33,593,329	41	41
AVRX-8	SEP2-CT8.1_80	28,294,978	42	43
AVRX-9	SEP2-CT7.7_10	29,927,066	43	43
AVRX-10	SEP2-CT7.7_11	27,023,641	43	43
AVRX-11	SEP2-CT7.7_12	31,302,866	42	42
AVRX-12	SEP2-CT7.7_13	26,791,740	43	44
AVRX-13	SEP2-CT7.7_46	33,106,734	42	42
AVRX-14	SEP2-CT7.7_48	27,834,839	45	46
AVRX-15	SEP2-CT7.7_85	27,648,758	43	43
AVRX-16	SEP2-CT7.7_86	30,032,397	43	43
AVRX-17	SEP2-Hg8.1_19	29,059,690	43	43
AVRX-18	SEP2-Hg8.1_20	32,381,027	44	44
AVRX-19	SEP2-Hg8.1_21	24,445,510	43	45
AVRX-20	SEP2-Hg8.1_24	30,206,664	42	43
AVRX-21	SEP2-Hg8.1_25	31,415,272	43	43
AVRX-22	SEP2-Hg8.1_55	24,646,853	43	43
AVRX-23	SEP2-Hg8.1_56	30,177,602	42	42
AVRX-24	SEP2-Hg7.7_28	28,964,924	42	45
AVRX-25	SEP2-Hg7.7_30	39,767,058	42	41
AVRX-26	SEP2-Hg7.7_31	26,843,136	41	42
AVRX-27	SEP2-Hg7.7_32	44,104,080	41	41
AVRX-28	SEP2-Hg7.7_68	25,479,196	41	41

Pre-assembly processing

The same pipeline of bioinformatic analyses (Figure 2) was carried out for rnasep1 and rnasep2, using the IFB-core high-performance computing cluster of the Institut Français de Bioinformatique (IFB) (ANR-11-INBS-0013). The raw data quality was assessed using FastQC (fastqc_v0.12.1) (Andrews, 2010) in combination with MultiQC (multiqc_v1.14) (Ewels et al., 2016). Fastp (fastp_v0.23.1) (S. Chen, 2023) was used to filter out adapter sequences as well as low-quality reads (PHRED score ≤ 30), and retrieve only post-filtering reads longer than 120 bp.

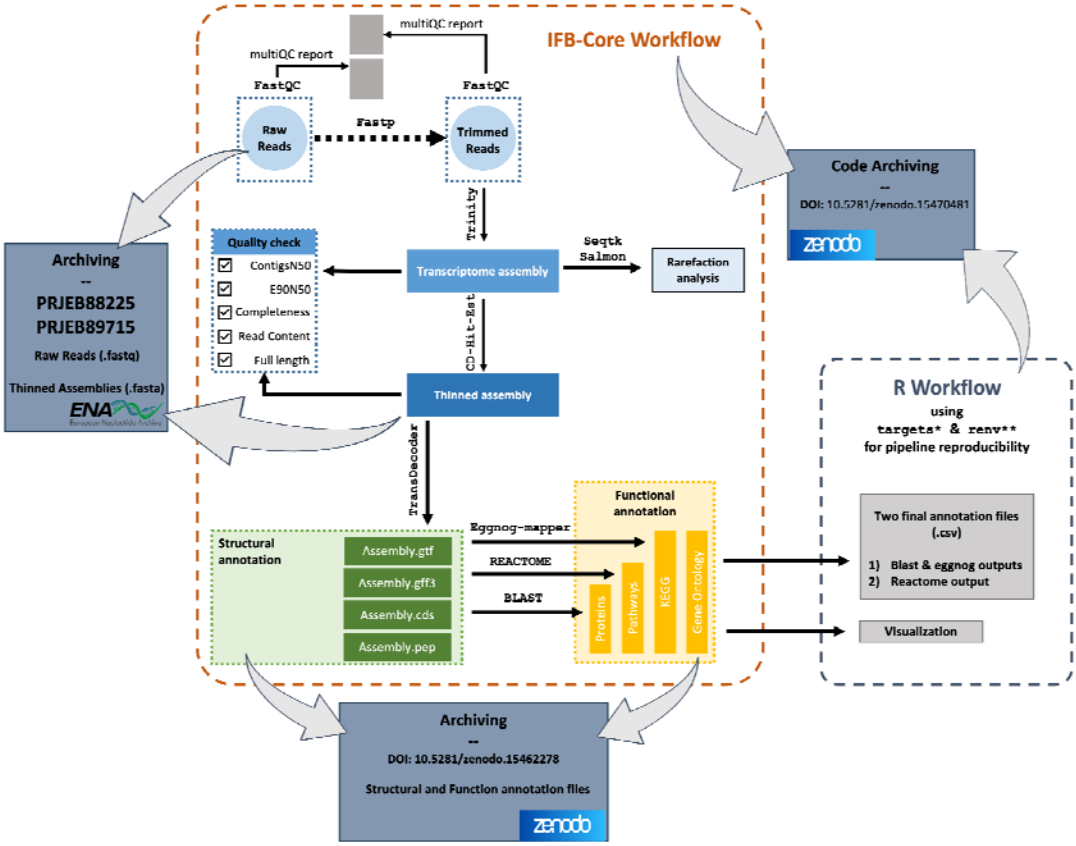

Figure 2 - Bioinformatic workflow from raw data to final assembly and annotation files. *(Landau, 2021); **(Ushey & Wickham, 2025)

De novo transcriptome assembly and thinning

Eighteen samples (n=3 per condition) and 16 samples (n=4 per condition) were randomly selected to be included in the rnasep1 and rnasep2 assemblies, respectively, to reduce the time and resource consuming. The *de novo* transcriptome assembly was performed using Trinity (trinity_v2.15.1) (Grabherr et al., 2011) with default parameters and *in-silico* normalization.

Assembled isoforms were clustered based on their similarities (default threshold: 95%) using the Cd-hit tool (cd-hit_v4.8.1) (Li & Godzik, 2006) to retain only the more representative sequences, thereby reducing transcriptome redundancy likely to result from the Trinity method. Finally, the putative coding regions (open reading frames [ORFs] ≥ 100 amino acids) were identified by Transdecoder (transdecoder_v5.7.0) (Haas, n.d.) enhanced by homology search using Blastp (blast_v2.16.0) (Camacho et al., 2009).

Assessment of the transcriptome assemblies

Several metrics were used to fully assess the quality of the assembly. First, TransRate (transrate_v1.0.3) (Smith-Unna et al., 2016) retrieved general statistics from the raw and thinned assemblies. Then, the contigs N50 statistic (at least 50% of the assembled transcript nucleotides are found in contigs that are at least of N50 length), based on the longest isoform per gene and the E90N50 statistic (N50 for top 90% most highly expressed genes) were computed using dedicated Trinity utilities and Kallisto (kallisto_v0.46.2) (Bray et al., 2016). BUSCO (Busco_v5.5.0) (Manni et al., 2021) was used to evaluate the completeness of the transcriptome

against two ortholog databases: Metazoa (metazoan_odb10) and Mollusca (mollusca_odb10). The full-length transcript analysis provided by Trinity was also performed using BLAST (blast_v2.16.0) (Camacho et al., 2009) and STAR (star_v2.7.11a) (Dobin et al., 2013) was used to align back the reads against the assembly to assess its read content.

To confirm that the reduced number of samples still ensure a sufficient read depth to catch all available transcripts, we performed an *ad hoc* rarefaction analysis as follows. For each assembly, all the input R1 and R2 fastq files were concatenated, then reads were sub-sampled (keeping pair information) using seqtk (seqtk_v1.3). (https://github.com/lh3/seqtk). For newly-hatched individuals, 1 to 80 million read pairs were sub-sampled. For one-month-old juvelines, 1 to 300 million read pairs were sub-sampled. Then, Salmon (salmon_v1.10.2) (Patro et al., 2017) was used to index Trinity assemblies and quantify the number of reads from each corresponding sub-sample that matches each Trinity transcript. Finally, the number of Trinity transcripts matched by at least 1 read was reported for each sub-sample.

Transcriptome annotation

Transcriptome annotation was performed stepwise based on the putative ORFs identified by Transdecoder. First, blastp and blastn searches were carried out against the UniProtKB/Swiss-Prot (Bateman et al., 2023) database using an e-value threshold of 1e-5, retaining only the best hit per query. The eggNOG-mapper v2 (eggnog-mapper_v2.1.12) (Cantalapiedra et al., 2021) tool, which performs functional annotations based on precomputed orthologous groups, was used to complete the Blast annotation with hits from the Pfam (Mistry et al., 2021), COG (Tatusov et al., 2000), KEGG (Kanehisa et al., 2017) and GO (Ashburner et al., 2000) databases.

Comparison between newly hatched and one-month-old juveniles

The ORF-related transcripts predicted in each assembly were compared to each other by a reciprocal blastn (blast_v2.16.0) (Camacho et al., 2009); the number of transcripts unique to each dataset and shared between datasets were represented using the VennDiagramm R package (H. Chen & Boutros, 2011).

Results

Illumina sequencing and pre-processing

The Illumina sequencing produced a total of 358,400,00 paired-end reads for newly hatched and 830,584,708 paired-end reads forone-month-old juveniles, with a mean length of 150 bp in both runs (see Tables 1 and 2 for sequencing statistics). The pre-processing resulted in 350,000,000 and 1,108,000,000 cleaned reads accounting for 48.8% and 66.7% of the raw data for newly hatched and one-month-old juveniles, respectively.

De novo transcriptome assembly quality assessment

For newly hatched juveniles, the thinned transcriptome assembly contained 244,772 transcripts with an average length of 687 bp (Table 3). The quality of the assembly was validated thanks to several metrics. About 87% and 99% of the orthologs from the BUSCO Mollusca and Metazoa databases respectively matched the assembly. In addition, 1,374 proteins matched Trinity transcripts from 80% to 90% of their protein length and 5,174 proteins were represented by nearly full-length transcripts. Besides, 94.7% of the reads used in the assembly process mapped back to the transcriptome.

The one-month-old juveniles transcriptome assembly obtained after redundancy reduction comprised 397,569 transcripts with a mean length of 577 bp (Table 4). A high proportion of

conserved orthologs from the BUSCO Mollusca (87.2%) and Metazoa (98.2%) databases were found in the assembly. Moreover, 1,451 proteins matched Trinity transcripts from 80% to 90% of their protein length and 5,271 proteins were represented by nearly full-length transcripts. Finally, 95.8% of the reads used to build the assembly mapped back to it.

Rarefaction analysis showed that, for both dataset, the subset of individuals used for assembly is an acceptable compromise between exhaustiveness of transcript inventory and computational effort (Figure 3).

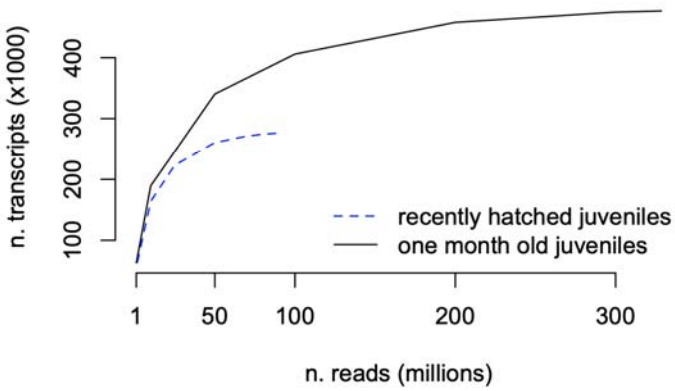

Figure 3 - Rarefaction curves performed on the raw Trinity assemblies.

Based on this assessment, we can assume that both assemblies are of good quality in terms of transcripts length and representation of conserved transcripts.

Table 3 - Transcriptome assembly and assessment metrics for Raw and Thinned assemblies for newly hatched juveniles

Type	Metric	Raw	Thin
Baseline			
	Number of Trinity "genes"	207672	204818
	Number of transcripts	282660	244772
	GC content (%)	36.4	36.13
	Smallest transcript	174	180
	Largest transcript	36551	36551
	Mean transcript length	741.29	687.16
	Bases with N (ambiguity)	0	0
	Proportion of N bases	0	0
	N50 (longest isoform per gene)	758	764
	E90N50	1932	1923
	Predicted number of transcripts with ORF	35681	26968
	Mean ORF (%)	49.43	47.97
Completeness			
	Mollusca_odb Complete and Single-copy (%)	43.9	55.3
	Mollusca_odb Complete and duplicated (%)	43.0	31.4
	Metazoa_odb Complete and Single-copy (%)	61.7	73.2
	Metazoa_odb Complete and duplicated (%)	36.2	24.7
FullLength			
	Number of proteins matching Trinity transcript from 80% to 90% of their protein length	1295	1374
	Number of proteins reprented by nearly full-length (>80%) transcripts	4901	5174
ReadContent			
	Aligned reads (%)	94.8	94.7

Table 4 - Transcriptome assembly and assessment metrics for Raw and Thinned assemblies for one-month-old juveniles

Type	Metric	Raw	Thin
Baseline			
	Number of Trinity "genes"	331430	325104
	Number of transcripts	483699	397569
	GC content (%)	36.94	36.65
	Smallest transcript	179	182
	Largest transcript	33040	33040
	Mean transcript length	621	577
	Bases with N (ambiguity)	0	0
	Proportion of N bases	0	0
	N50 (longest isoform per gene)	594	600
	E90N50	2005	1990
	Predicted number of transcripts with ORF	43409	31200
	Mean ORF (%)	49.5	48.5
Completeness			
	Mollusca_odb Complete and Single-copy (%)	38.9	51.8
	Mollusca_odb Complete and duplicated (%)	48.6	35.4
	Metazoa_odb Complete and Single-copy (%)	59.5	71.5
	Metazoa_odb Complete and duplicated (%)	38.8	26.7
FullLength			
	Number of proteins matching Trinity transcript from 80% to 90% of their protein length	1467	1451
	Number of proteins reprented by nearly full-length (>80%) transcripts	5388	5271
ReadContent			
	Aligned reads (%)	94.0	95.8

Transcriptome annotation

The structural annotation performed with Transdecoder on the thinned assemblies identified 39,804 and 49,402 ORFs for rnasep1 and rnasep2, respectively. These ORFs were submitted to functional annotation.

In the assembly performed from newly hatched individuals, Blast annotated 28,407 transcripts against the SwissProt/Uniprot database. In addition, 28,313 orthologous hits were found by eggNOG-mapper with 21,692 transcripts annotated with gene ontology terms (for the occurrence of the top 20 GO terms from each namespace: Figure 4a). Moreover, 19,471 transcripts were associated to KEEG KO and 10,775 to KEEG pathways (Figure 5a).

In the one-month-old assembly Blast, 32,746 transcripts were annotated against the SwissProt/Uniprot database. This annotation was completed using eggNOG-mapper. Overall, 32,259 orthologous hits were reported and 24,257 transcripts matched gene ontology terms (Figure 4b), 22,222 transcripts matched KEEG KO and 11977 matched KEGG pathways (Figure 5b).

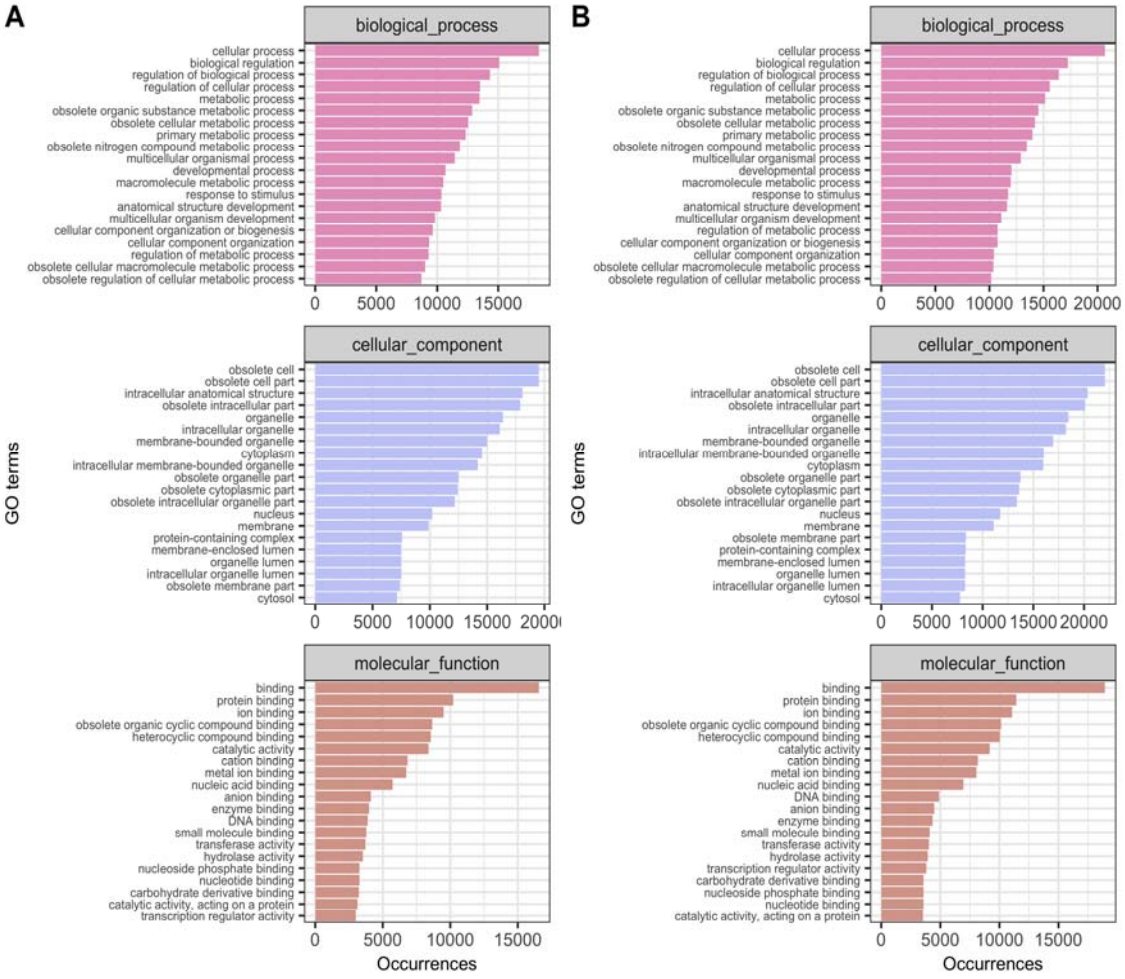

Figure 4 - Occurrence of the 20 top GO terms related to the putative ORFs by namespace for A) newly hatched and B) one-month-old juveniles.

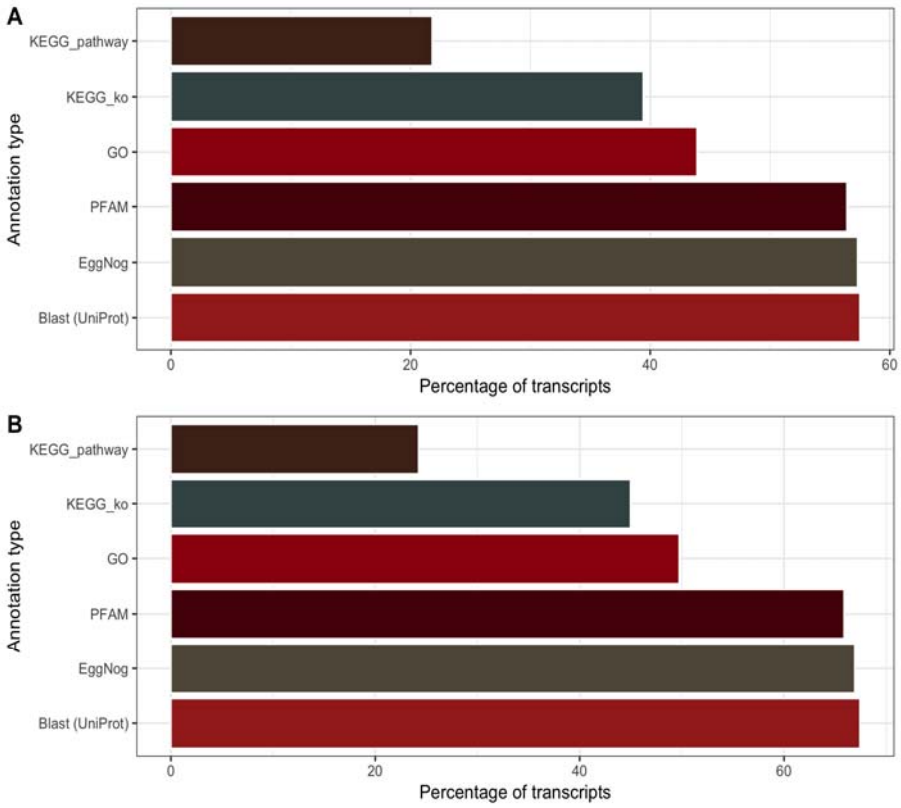

Figure 5 - Percentage of transcripts matching at least one entry from each annotation type for A) newly hatched and B) one-month-old juveniles.

Newly hatched vs one-month-old juveniles

Overall, 26,668 transcripts were shared between “rnasep1” and “rnasep2” assemblies, representing 68% and 54% of each assembly, respectively. Rarefaction analysis showed that the difference in the number of transcripts detected for newly hatched and one-month-old individuals is a biological signal and not an artifact due to uneven sampling (Figure 3 and 6).

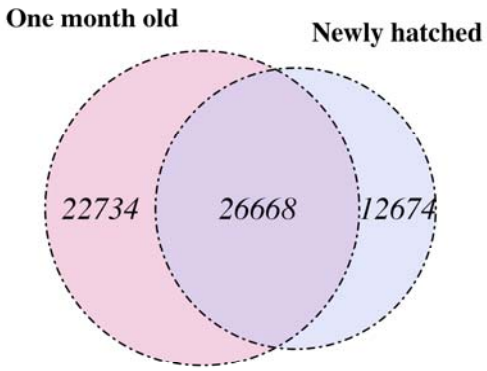

Figure 6 - Venn diagram displaying the number of transcripts shared or exclusives between both assemblies.

Conclusion

Both transcriptome assemblies significantly contribute to improve molecular resources for conducting genomic studies on Cephalopods, particularly *S. officinalis*. The overall overlap rate (43%) between newly hatched and one-month-old juveniles transcripts attests to the importance of considering developmental stages and tissues in transcriptomic studies, as about half of the detected transcripts are private to a given dataset. In this sense, the use of multiple tissues (whole body in newly hatched and heads in one-month-old juveniles), although it may obscure some tissue-specific patterns, provides valuable resources for organism-level investigations. Moreover, the inclusion of individuals from various metal and pCO_2 exposure conditions diversified the transcriptomic landscapes included in the assemblies, broadening their range of relevance. This is particularly critical in the context of global change which requires to consider the influence of combined environmental stressors.

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Conflict of interest disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data, scripts, code and supplementary information availability

All the transcriptomic data (raw reads and assemblies) are available at the European Nucleotide Archive under the accession numbers: PRJEB89715 (rnasep1) and PRJEB88225 (rnasep2). The annotation files of the two assemblies are available on Zenodo with DOI: https://doi.org/10.5281/zenodo.15462278. The Github repository containing the codes used for bioinformatic analyses was released on Zenodo, accessible under the DOI: https://doi.org/10.5281/zenodo.15470481.

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